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THE RELATION OF ACTIVE TO INACTIVE FIBRES IN FRACTIONAL CONTRACTION OF MUSCLE.

By SYBIL COOPER (*Research Fellow of St Hilda's College*).

(*From the Physiological Laboratory, Oxford.*)

Introduction.

The present problem arose out of the work of Cooper, Denny Brown and Sherrington(1, 2) on fractionation and occlusion. It was assumed that in a fractional isometric contraction the tension recorded was a true measure of the tension in the active fibres. If the active fibres are arranged "in series" along the axis of the muscle then this would be true; but if, on the contrary, they are "in parallel" at one end of the muscle then a wasteful stretch of the fibres at the other end must result, making the records not strictly isometric. Part of the present work has been an attempt to find what degree of waste this possible stretching might entail. Isometric and isotonic records have been taken, as a contraction in an intact animal lies somewhere between these. Stimulation has been both tetanic and by single break shocks, for the latter help to throw light on the beginning of tetanus. In default of a convenient muscle in which the contraction can be confined definitely by experiment to one end, the double preparation of the frog's semimembranosi has been used. This can be arranged so that one muscle, when it contracts, can either pull directly on a fixed support or can pull, and thus stretch, the other muscle. A comparison of the two results shows a certain loss of effectiveness in the second arrangement.

In the later part of the paper this problem has been approached from a different angle. The fractional contractions, in response to reflex stimulation, of certain muscles in the cat have been studied and some conclusions reached on the region of a fractional contraction and the relation of this region to the nerves supplying it.

I. Experiments on frog's muscles.

A. *Method.*

In these experiments the two semimembranosi were finally chosen as suitable muscles. They were dissected out carefully on each side with

their nerve supply intact; the whole upper sciatic was prepared and no attempt made to isolate the nerves to the muscles. The knee joints were left attached to the muscles in order to take steel hooks; and the ischio-pubic part of the pelvis girdle was kept to form a junction between the two muscles. The acetabula were also left so that they could if necessary take a small clamp to fix the pelvis. This gives a double muscle preparation that may be supposed to be one long muscle divided into two parts. By stimulating both nerves together the whole preparation can be made to contract; by stimulating one nerve only, then one muscle will contract and, if the pelvis is free, it will stretch the other muscle.

The stimulation throughout was from a class coil with two volts in the primary circuit, the muscle being either tetanised or subject to single break shocks applied indirectly through the nerve; direct stimulation seemed more detrimental to the muscles. Various ways were tried of fixing up the muscles, and finally it seemed most satisfactory to set up the preparation over, but not touching, damp filter paper; owing, however, to this exposed position the experiments had to be carried out as quickly as possible. Summer frogs gave the best results. Isotonic and isometric records were taken on a smoked drum.

For the sake of convenience the two muscles have been called α and β; α being the one nearer the recording lever. The contractions have been called A, B, C; A being a contraction of α alone with the pelvis fixed, B signifying a contraction of α with the pelvis free, and C being a simultaneous contraction of both muscles.

B. *Results.*

(a) *Isotonic tetanus.* In these experiments the double preparation was attached to an isotonic lever which registered rectilinear ordinates. The records were taken with a varying afterload; and the resting muscle was just not slack. The best procedure was to take three readings for each load, viz. (1) A, (2) B, and (3) A again, then, if necessary, interpolating for the value of A finally taken; in this way A is comparable with B for each load. The true height and load were calculated and the product of these gave the work performed. Fig. 1 a shows the relation of the load to the work, the upper curve A being taken from the A contractions and the lower B from the B contractions. In the B contraction with a small load the α muscle can shorten almost freely at the tibial end so that the work done is about the same as for the A contraction. But if the load is increased, then, since α is not fixed at the pelvis but at the tibial end of β, β begins to act like a piece of elastic and yields as α

contracts. Thus, though α has received the same stimulus as in A, its work is now divided between lifting the load and stretching β; only that

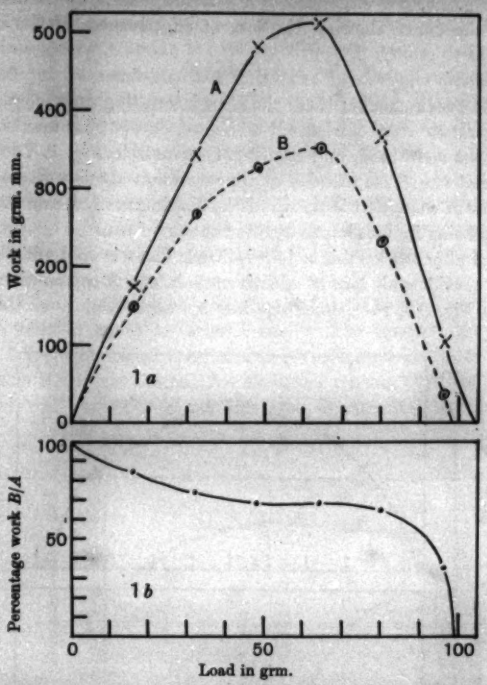

Fig. 1 *a*. Comparison of the work done by a frog's semimembranosus, during short tetanic isotonic responses with varying load, *A* when the muscle is held rigidly at its pelvic end, *B* when the muscle is connected through the pelvis to the other unstimulated semimembranosus.

Fig. 1 *b*. Expresses the work in *B* as a percentage of the work in *A* for the same load. Temperature 19° C.

part of the work due to lifting the load can be calculated from the height, so that in *B* the work recorded is less. As the load is increased more and more work is expended on stretching β, so that finally α, though subject to a maximal contraction, will not lift the load at all, but will only shorten at the pelvic end. This point is reached at a load of about

98 grm. in Fig. 1 *a*, whereas in the *A* contraction the muscle just fails to lift 105 grm. In other experiments the difference was greater than this. In Fig. 1 *b*, *B* is expressed as a percentage of *A* as calculated from Fig. 1 *a*, the curve showing the loss of effectiveness of the α muscle when it has to stretch β.

(*b*) *Isotonic single break shocks.* A few experiments have been done with single break shocks; these show an interesting difference of effect. The experiments were carried out as before, except that maximal single break shocks were used, and Fig. 2 corresponds to Fig. 1. The striking point is that the *B* contraction at its maximum shows only about half the amount of work that is shown at the maximum of *A*, and at the load when *A* is doing its maximum amount of work *B* cannot lift the weight; finally in *A* about twice this weight is lifted. There is no doubt that there is a very considerable loss of effectiveness in the *B* contraction and, as seen from Fig. 2 *b*, the percentage loss increases rapidly as the load is increased.

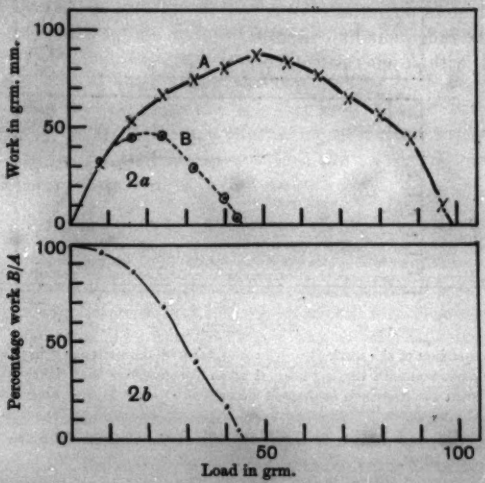

Fig. 2 *a*. Comparison of the work done by a frog's semimembranosus, during break shock isotonic twitches with varying load, *A* when the muscle is held rigidly at the pelvic end, and *B* when the muscle is connected through the pelvis to the other unstimulated semimembranosus.

Fig. 2 *b*. Expresses the work in *B* as a percentage of the work in *A* for the same load. Temperature 18° C.

In a *B* contraction if the α muscle has to lift anything but a small load it will, owing to the afterloading, first stretch β. It is only when the force exerted on β is the same as the force necessary to lift the load that the load will be raised. In a twitch this force may not be developed by the time the contraction is over, so that the load is never lifted. If the contraction in α causes a rapid stretch of β, then β may act to some extent as a partial release to α, cf. Gasser and Hill(3): the force exerted by α will be reduced, and there may not be time, in the twitch, for it to rise again to the value necessary to lift the load.

(*c*) *Isometric tetanus.* The isometric records were all taken with a small torsion wire myograph with a straw lever capable of writing on a smoked drum. Several hundred grammes tension could be recorded and the tendon movement was magnified ten to fifteen times. The preparation was attached by a steel hook through one tibial tendon to a stand that had a fine screw adjustment for horizontal movement, so that the tension could be altered easily. The tension developed for varying degrees of initial tension was investigated in the two cases *A* and *B*. The range was limited, for an initial tension over about 50 grm. combined with a maximal tetanus was detrimental to the muscle;

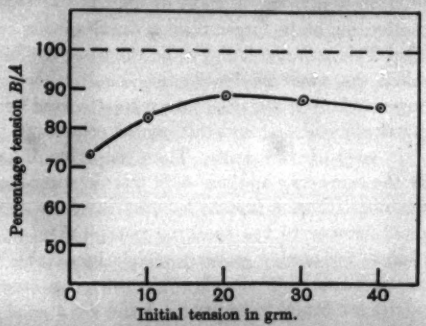

Fig. 3. Comparison of the final tension developed in the frog's semimembranosus, during short isometric tetanic responses with varying initial tensions, *A* when the muscle is fixed at the pelvic end, and *B* when the muscle is connected through the pelvis to the other unstimulated semimembranosus. The final tension in *B* is expressed as a percentage of that in *A* for the same initial tension. Temperature 17° C.

Evans and Hill(4) showed that the maximum tensions developed in an isometric contraction in a frog's muscle were obtained with only moderate initial tensions. It was best to take readings for *B* before

and after those for *A* and to interpolate where necessary. Final tensions of 100 to 150 grm. were developed. The loss of effectiveness can again be shown by expressing the final tension developed for the *B* contraction as a percentage of that for *A*, as is done in Fig. 3. In this figure when a certain degree of initial tension has been reached, *i.e.* when the contraction is more truly isometric, the effectiveness of *B* reaches about 90 p.c. that of *A*; about this figure has been reached in all the experiments. Therefore where half the preparation is inactive the active muscle loses about 10 p.c. of its effectiveness; the loss has never been found to be much less than this with initial tensions varying between 10 and 50 grm.

Two other points have been investigated. First the relation between the maximal contractions of the α and β muscles alone and together. Here it was found in a good preparation that the tension developed by α in an *A* contraction was equal to that developed by β under similar circumstances, and that this tension was also equal to that of α and β together in a *C* contraction. Secondly, some measurements were taken of the *C* contraction when the muscles contracted together and when α contracted first and stretched β and then β contracted. It was thought that β might be in a favourable state of high initial tension and the double contraction might be larger than a simultaneous contraction of the two muscles. This was not found to be the case, the successive contractions reached the same tension as the simultaneous ones, so that there is no reason to believe that the intact muscle would gain anything by contracting at one end first and then at the other.

(*d*) *Isometric single break shocks.* These experiments were carried out in exactly the same way as those with tetanic stimuli and isometric recording; the initial tension had to be very carefully controlled as it makes a great difference to the resulting tension (Doi(5), Fulton(6)). The preparation is reasonably stable to single break shocks so that it was possible to take a series of records of *A* at varying tensions, followed by a similar series for *B* and to return to *A* and get a good control. The results of an experiment are expressed in Fig. 4, again giving *B* as a percentage of *A* as in Fig. 3. The initial tension was increased up to 140 grm. and the final tensions were still increasing, but the series were not carried further for fear of permanently damaging the muscles. Even at an initial tension of 140 grm. the final tension of the *B* contraction was 6 p.c. less than that of *A*; and at 40 grm. the loss of effectiveness of α was 15 p.c. (cf. 10 p.c. in isometric tetanus).

II. Observations on muscles in the cat.

The results so far show that if active muscle fibres are arranged in series with inactive ones, then the latter are responsible for a loss of

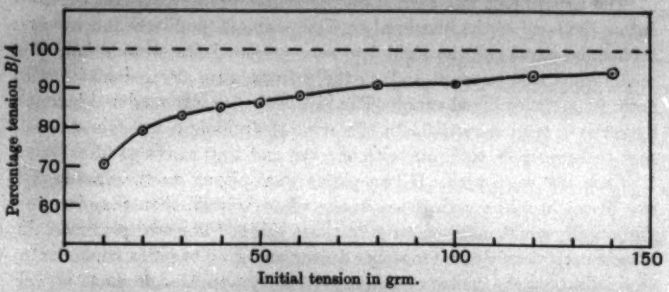

Fig. 4. Comparison of the final tension developed in a frog's semimembranosus, during break shock isometric twitches with varying initial tensions, *A* when the muscle is fixed at the pelvic end, and *B* when the muscle is connected through the pelvis to the other unstimulated semimembranosus. The final tension in *B* is expressed as a percentage of that in *A* for the same initial tension. Temperature 18° C.

effectiveness of the former. It now remains to be seen whether in parallel fibred muscles the innervation is such that only one end of the muscle contracts in a minimal contraction or whether the loss of effect that would thus follow is overcome by a more economical means whereby the active and inactive fibres are arranged in parallel.

Most muscles in the cat have a tendon arrangement that prevents loss of effectiveness in a fractional contraction, but some muscles remain something of a problem. The tenuissimus and sartorius, for example, are made up of short spindle-shaped fibres dovetailed together and lying parallel to the long axis of the muscles. No tendinous network or fine threads of tendon have so far been seen in the muscles, though possibly sufficiently discriminating methods of observation have not been used. If these long thin muscles contract only at one end in a fractional contraction, then it seems inevitable that they should have stretched fibres at the other end.

(*a*) *Tenuissimus.* The tenuissimus of the cat is a long muscle of very small cross-section. Sherrington(7) showed that it takes part in the flexion reflex, and Graham Brown(8), Porter and Hart(9) and Adrian(10) have given descriptions of it. The nerve supply is near the

centre, generally 5 or more cm. from either end, and bifurcating just before entering the muscle. The muscle fibres are only about 2 cm. long(10). The muscle can fairly readily be exposed for almost its entire length and is therefore easy for direct observation.

The experiment was carried out on a decerebrate cat with the cord cut at the level of the first lumbar. The peroneal, popliteal and internal saphenous nerves of the right leg were prepared for stimulation. The tenuissimus was exposed and partially freed, care being taken not to harm its nerve or blood supply. The rest of the leg was rendered inactive by nerve or tendon section. In observing the muscle it was found necessary to have spots to fixate with the eye and tiny pieces of filter paper or black silk were used. If two pieces were placed on the muscle and two pieces in a line with these on the tissue behind, then the pieces on the muscle would move relative to those behind. The two pieces on the muscle very clearly came together during any kind of reflex contraction, especially near the centre of the muscle. They appeared to do so as well for a weak reflex contraction at the ends of the muscle, but it might be questioned whether the contraction was a true one and not a mechanical effect due to the active pull of the central part of the muscle. A much more satisfactory result was obtained by another method; a small piece of cork was placed under the muscle at any desired spot and fixed to the tissue underneath by hedgehog quills, then the connective tissue at the extreme edges of the muscle was fastened to the cork by quills. By this means the nerve supply and muscle substance were unharmed, but the muscle was given a local fixed point so that the muscular tissue on one side of the fixed point could not be affected mechanically by that on the other side. The cork was first put about 2 cm. below the point of entry of the nerve and the leg held partially flexed, so that the muscle was slack. On stimulation of the central end of the peroneal nerve (class coil, 2 volts in the primary, make and break shocks) the threshold for reflex contraction of the muscle was found to be 32 cm. and there seemed to be a trace of contraction in the whole length of the muscle. At 30 cm. there was no doubt about the contraction, the part of the muscle below the cork could be seen and felt getting taut and all the slack part was gathered up. This was true for all strengths greater than threshold; if the part near the nerve contracted then that beyond the cork did so as well. The internal saphenous nerve was also used for afferent stimulation as segmentally it would be a little further removed from the tenuissimus centre than the peroneal nerve. The threshold was about 28 cm., and at 27 cm. both parts of the muscle

undoubtedly contracted. The cork was then moved to 4 cm. beyond the nerve and about 2·5 cm. from the lower end of the muscle; the above observations were repeated with exactly the same results.

Adrian proved, by counting the fibres in the main nerve trunk and the branches, that the individual fibres in the nerve to tenuissimus divide and send branches to both parts of the muscle; this is of importance in consideration of muscle economy, for it means that both halves of the muscle will contract together. The present observation carries the result a stage further, for it leads to the conclusion that these nerve branches each supply a chain of muscle fibres rather than a number of them side by side. In a fractional contraction therefore both halves of the muscle contract and each half contracts throughout its length; a larger contraction means that more parallel chains are brought into action. Thus there should be no loss of effectiveness in a submaximal contraction.

(*b*) *Sartorius.* The sartorius in the cat is a long thin band-like muscle stretching from the thigh to the knee, the fleshy external band being inserted into the patellar tendon and the broad thin part into the tibia on the medial side of the leg. The cats were prepared as described in the previous section save that the nerve to the sartorius was left intact. The muscle was watched during reflex contractions set up during stimulation, at a strength near threshold, of afferent nerves in the ipsilateral leg. It is much more difficult in a wide muscle to be sure of the point of contraction, but it was clear that the contraction was never confined to one end of the muscle, though at times it was certainly confined to one side of the muscle. The results in Table I were obtained from various nerves.

TABLE I.

Cat	Afferent nerve	Threshold for reflex contraction cm.	Results
1	Popliteal	24	Whole muscle contracts
	Peroneal	22	,, ,,
	N. to hamstrings	23	Tibial strip contracts
	N. to hamstrings	19	Patellar strip contracts
	Internal saphenous	37	,, ,,
2	Internal saphenous	36	Patellar strip contracts
	N. to quadriceps	37	Whole muscle contracts, but patellar strip most
	Ext. cutaneous	30	Whole muscle contracts

The nerve supply to sartorius consists of two nerves that come from the main femoral nerve; these two nerves may go to the muscle together, or one may run with the internal saphenous nerve for 2 cm. or more and

then go across to the sartorius. The upper of these nerves always supplies the patellar strip of the muscle as can be proved by getting a reflex contraction before and after cutting this branch. The arrangement of these two nerves is of interest and can be easily studied if the muscle is stripped off and the back examined. The upper one goes straight across the muscle, only held to it lightly by connective tissue, and does not enter the muscle until it reaches the fleshy band; the lower one begins to send off branches as soon as it reaches the muscle. These branches very obviously run from the nerve down towards the knee, each branch being joined by blood vessels, and the back of the muscle may look like a piece of material with fine pin stripes. If each branch is followed back to the main nerve there is generally found a very fine thread of nerve leaving the main branch and turning up to the thigh, as is shown in Fig. 5. If the nerves to the muscle are cut and a branch pinched before

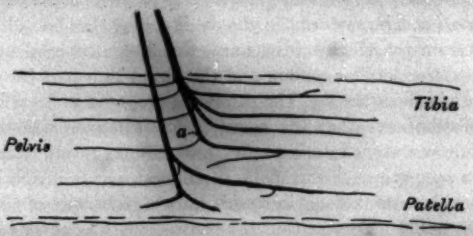

Fig. 5. Sketch of part of the under surface of a cat's left sartorius muscle showing the nerve supply. Note the branching of the nerve trunks at points well before the nerve enters the muscle; it is at points such as the one marked *a* that the fibre divisions of Fig. 6 are found.

the fine thread comes off, a narrow strip contracts the whole length of the muscle. Also if, after the main branch has been cut, one of the divisions is cut and stimulated centrally, then the part of the strip supplied by the other division contracts. This suggests an axon reflex and leads to the supposition that the nerve fibres actually divide in much the same way as they do in the nerve to tenuissimus.

In order to see the actual fibre divisions, pieces of sartorius nerve were taken, just central to the point where the fine branches were given off to the upper part of the muscle, and stained in bulk in osmic acid. These pieces were later teased out in glycerine and examined microscopically. In nearly every specimen examined there was at least one nerve fibre division, and on one slide there were no less than nine divi-

sions. Most of the fibres divided into two, but there was one case where the parent fibre very clearly broke up into three, as shown in the micro-photograph in Fig. 6 *a*. This kind of division is probably common enough when the nerve has entered the muscle, but here it is taking place a whole centimetre or more outside in the nerve trunk. Fig. 6 *b* is from

Fig. 6. Branching medullated fibres in nerve trunk to cat's sartorius. Osmic preparations magnified 266 times. (*a*) shows an unusual division into three, (*b*) a more common division into two.

the same slide and shows a more usual branching into two. Table II gives the diameters of all the dividing fibres on that slide.

TABLE II.

No. of division	Parent fibre μ	Branch 1 μ	Branch 2 μ	Branch 3 μ	Remarks
1	13·2	12·0	8·0	8	See Fig. 6 *a*
2	4·8	3·6	5·2	—	
3	16·0	13·2	7·2	—	
4	16·0	14·0	7·2	—	
5	16·8	15·2	9·2	—	See Fig. 6 *b*
6	20·0	12·8	8·0	—	
7	16·0	16·0	10·0	—	
8	8·0	6·0	4·0	—	
9	6·0	7·2	4·0	—	
10	24·0	16·0	14·0	—	Another nerve

Degeneration experiments indicate that the majority at least of such dividing fibres are motor. Also whereas it is an advantage for a strip of muscle to contract from end to end, there would be loss of sensory appreciation if one afferent fibre had its origins over a large area.

III. Remarks on the structure of muscle.

Beritoff(11) and Lindhard(12) have recently published accounts of frog's muscle structure, the former giving a good summary of the earlier work. The frog's sartorius is usually taken as a typical parallel-fibred muscle, but a closer examination of its structure reveals that the fibres stretch only a half to two-thirds of its length. They are not fastened together, but show a dovetailing arrangement. One end of each fibre is attached to one of the tendons and the other end tapers and is met by a minute thread coming to it from the other tendon. The more complicated muscles have oblique fibres, but seemingly in every case there is either a tendon aponeurosis round one end of the muscle, or a strong band of tendon going up into the muscle, so that each fibre has very clearly a double attachment to tendon tissue. Of this tendon tissue it cannot perhaps be said that it is inextensible, but compared with the muscle fibres it is practically so. Thus each fibre when it contracts contributes directly to the shortening or increase of tension in the muscle and any recorded tension is a true expression of the tension in the active fibres.

When the very much longer muscles of mammals are considered, the comparatively short length of the fibres seems to demand a great deal of tendinous tissue, but often here some kind of herring-bone arrangement takes place, so that many fibres can share two tendon threads. When it is argued that there is no tendinous tissue in the muscle, a possible fallacy arises in the method of examination. Nitric acid is often used in the preparation of the muscle, regardless of the fact that it destroys tendinous tissue; if then the tissue is not there finally it is no proof that it was not once there. Many muscles, therefore, whose fibres do not stretch the whole length, probably have a fine net sent up from each tendon. If the tendinous net is really absent in some parallel-fibred muscles, then it seems likely that the fibres are attached to each other in chains the whole length of the muscle; in a fractional contraction a number of these chains will contract.

Conclusion.

If a muscle gave only a local contraction at one end during a fractional response, it would do so with a loss of effectiveness to the muscle as a whole. Extra fibres would probably have to contract in order to counteract the stretching of the inactive fibres. The muscle seems to overcome this difficulty in two ways. Firstly, the nerve supply is so

arranged that in parallel-fibred muscles such a local contraction never takes place; in a submaximal contraction a chain of fibres is put into action from end to end of the muscle, and a bigger contraction means a greater number of chains. Secondly, the tendons and tendinous tissue are so arranged that each fibre has a double connection and thus has a chance to register its contraction without loss of effectiveness.

SUMMARY.

1. An attempt has been made to identify the relation of the active fibres to the inactive in a fractional contraction of a muscle.

2. Isometric and isotonic experiments are described on the double semimembranosus muscles of the frog. One of these muscles represents active fibres and the other inactive fibres in series and liable to be stretched. In an arrangement such as this the inactive fibres are always found to be responsible for a loss of effectiveness of the active ones, so that this would not be an economical relation for the fibres in a muscle.

3. Reflex contractions were observed in the tenuissimus and sartorius of the cat. In these muscles a very small fraction was made to contract and there was always evidence that a strip contracted along the whole length of the muscle. A study of the nerve supply to the sartorius helped to show how this was possible, and a description is given of nerve fibres branching in the nerve trunk to supply different ends of the muscle.

4. The tendon arrangement of muscles is discussed.

I wish to thank Sir Charles Sherrington for his helpful advice and encouragement throughout the work.

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A NOTE ON THE EFFECT OF SODIUM SULPHATE ON THE OXYGEN USAGE OF THE KIDNEY.

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Barcroft and Brodie(1), Barcroft and Straub(2) and Knowlton and Silverman(3), using the technique developed by Barcroft and his associates for determining the gaseous metabolism of an organ, found an increase in the oxygen consumption of the kidney during sulphate diuresis. Bainbridge and Evans(4), using the method of Evans(5), also found (one experiment) an increased oxygen usage during sulphate diuresis. On the other hand Tamura and Miwa(6) could detect no appreciable change. Recently Hayman and Schmidt(7) repeated the work of Barcroft and Brodie and found that sulphate diuresis was not necessarily accompanied by an increase in oxygen consumption although a profuse diuresis associated with an increase in the renal blood flow was usually accompanied by an increase in the renal oxygen consumption. If the blood flow was controlled no increase followed.

In a series of eight experiments, using the same method as Hayman and Schmidt, I have obtained similar results; viz., no appreciable increase in the oxygen usage of the kidney after the intravenous injection of sulphate unless a marked increase in the renal blood flow and a profuse diuresis occur. In one experiment the blood pressure was reduced to 30 mm. of Hg in order to stop the formation of urine, and although the injection of 50 c.c. of 10 p.c. sodium sulphate solution raised the renal blood flow from 27·3 to 52·1 c.c. per min. the oxygen consumption remained constant to within 10 p.c. (0·067 c.c. per grm. of kidney tissue per min.). This is in agreement with the observations of Bainbridge and Evans(4) that the gaseous metabolism of the kidney is not altered by changes in the rate of blood flow through the organ.

Further evidence on this question was obtained by determining the oxygen consumption of the isolated kidney perfused with the heart-lung preparation, using the method of Fee and Hemingway(8). In a series of eight experiments the addition of amounts up to 2 grm. of

sodium sulphate to a litre of circulating blood did not alter the (diastolic volume) : (oxygen consumption) ratio of the heart-lung preparation more than 5 p.c. Usually a slight rise (about 2 p.c.) occurred in the oxygen consumption for a given diastolic volume. A typical experiment is given in Table I. Fifteen experiments were then performed with the

TABLE I. Heart-lung preparation; closed circuit. 600 c.c. blood in circulation. Sodium sulphate added as indicated.

Time	Diastolic volume $X+\ldots$ c.c.	Cardiac O_2 usage c.c./min.
0′ 0″– 3′ 8″	3·5	12·99
2 grm. sodium sulphate added to blood.		
6′ 11″– 9′ 16″	3·7	13·11
9′ 16″–12′ 24″	3·5	12·99
1 grm. sodium sulphate added to blood.		
15′ 27″–18′ 35″	3·4	13·11

closed heart-lung-kidney preparation in order to determine the renal oxygen consumption during sulphate diuresis. It was found that whereas the addition of 2 grm. of sodium sulphate to the circulating blood would increase the output of urine from five to ten times and also increase the percentage and absolute amounts of sulphate excreted, no appreciable change in the oxygen consumption of the kidney took place. In all these experiments the renal blood flow was between 75 and 130 c.c. per min. and was practically unaltered by the addition of sulphate to the blood. This point was also noticed by Bainbridge and Evans(4). Arterial pressure was carefully controlled. Data compiled from a typical experiment is given in Table II. The values of renal

TABLE II. Closed heart-lung-kidney preparation. Approximately 1000 c.c. blood in circulation. Blood temperature 36·5–37·0° C. Renal blood flow during determinations 125 c.c. per min. Kidney weight 33·0 grm. 2 grm. sodium sulphate in 10 c.c. saline added to blood at 4.05. Absolute output of sulphate in urine increased approximately six times at height of diuresis. Experiment terminated by œdema 20 min. after last readings given.

Time p.m.	Urine c.c./10 min.	Renal O_2 usage c.c./min.	Renal blood pressure mm. Hg
3.41–4.00	0·3	1·93	103
4.00–4.09	0·3	2·07	110
4.09–4.16	2·1	1·87	111
4.16–4.25	2·2	1·61	112
4.25–4.30	4·0	1·71	110

oxygen consumption given for each period are the average of two or more separate determinations made during that period.

It would appear, therefore, that the actual excretion of large amounts

of sulphate by the kidney is not attended with an increase in the renal oxygen consumption unless there is a concomitant increase in the renal blood flow. The actual relations existing between the volume of urine excreted, the renal blood flow, and the oxygen usage of the kidney are, however, impossible to determine with the existing rough methods of measuring renal metabolism.

The expenses of this investigation were defrayed by a grant from the Royal Society. My thanks are due to Mr G. Pearson for valuable assistance with the operations and analysis.

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612.880.794:615.785.1–092.259

THE ACTION OF STRYCHNINE ON SENSORY END ORGANS IN MUSCLE AND SKIN OF THE FROG.

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Strychnine has such a profound effect upon reflex activity that it is of some interest to enquire into its influence upon the discharge of impulses from the peripheral sense organs. There are, of course, many reported observations as to the action of this drug upon organs of special sense. They are, however, conflicting, many of them being subjective, and the recent work of Adrian and Matthews(1) on the impulse discharge from the eye of the Conger eel makes it likely that here the effect of strychnine is due to its action on the synaptic connections of the retina rather than on the excitability of the light receptors. On the other hand, Lapicque(2) has shown that strychnine shortens the chronaxie of motor nerve fibres and Danilewsky and Perichanjanz(3) that it increases the irritability of motor nerve fibres: it is unlikely therefore that it should be entirely without effect on the sensory end organ.

The methods recently developed for recording the discharge of impulses from sense organs make it a fairly simple matter to study the action of drugs upon them. In the present work the discharge of proprioceptive impulses from a muscle under tension has been recorded before and after strychnine, and the impulses from strychninised frog skin have been studied by way of comparison.

The preparation employed was the excised muscle *flexor superficialis digitorum* with the connecting nerve *tibialis superficialis* of medium-sized frogs (*R. temporaria*). The muscle was left attached to the tibia and thereby mounted in one compartment of a double moist chamber. This compartment was then partly filled with Ringer fluid or Ringer-strychnine fluid so that the muscle was bathed with the desired solution. A thread from the tendon of the muscle was connected to a loading lever on which was hung a weight that was lowered at a constant rate by an oil dash-pot. The nerve was so arranged as not to come in contact with the fluid bathing the muscle and was suspended between two non-polarisable electrodes in the second compartment of the moist chamber.

Connections from these two electrodes led to the three valve amplifier and capillary electrometer previously described by Adrian(4). The strychnine solutions were made up in frog's Ringer with either strychnine sulphate or strychnine nitrate.

The observations completely confirmed the earlier work of Adrian(4) and Adrian and Zotterman(5) as regards the nature of the discharge of afferent impulses from a stretched muscle. On applying the load the frequency of impulses rapidly rose to a maximum and then as a result of adaptation of the end organs fell more slowly to a level that was maintained indefinitely. By keeping the muscle and nerve constantly moist, by carefully controlling the speed and amount of loading and by allowing equal intervals of rest it was possible to obtain consistent and reproducible results. The impulse frequency at any given time in the early course of a loading was usually found not to vary by much more than 10 p.c. from the frequency at a like time in similar stretches of the same muscle. With the stimuli in use the maximal frequency of the impulses was usually in the neighbourhood of 100–200 a second. The character of the discharge showed that a considerable number of sense organs was in action, but in the later stages when the frequency was lower it was often possible to make out a series of impulses occurring at regular intervals. In most of the work no attempt was made to restrict the number of end organs in action, since the impulse frequency was readily determined even at the height of the discharge. In some of the later experiments, however, a majority of the nerve fibres was transected by the method recently described(6) so that the remaining fibres conducted impulses from only a few end organs. This method has, in fact, proved to be a simple means for obtaining the discharge from apparently single sense organs.

The following procedure was usually employed. While still in Ringer's fluid the muscle was stretched at intervals of five to seven minutes and the impulses resulting from alternate loadings were photographed. In this way a representative series of discharges extending over a considerable period of time was obtained without exposing an unnecessarily large amount of photographic paper. A weak solution of strychnine was then substituted for the Ringer in the muscle chamber, the loadings were continued at the same intervals and the discharges accompanying every other stretch were recorded as before. This was continued until the strychnine had reached a concentration that checked the discharge of impulses or reduced it to a very low frequency. Fig. 1 shows four records chosen from a typical series in which the nerve had been partially

transected. They give only the beginning of the discharge *A* with the muscle still in Ringer; *B* 35 min. after immersion in 0·0001 p.c. strychnine; *C* after 19 min. in 0·001 p.c. strychnine, and *D* 27 min.

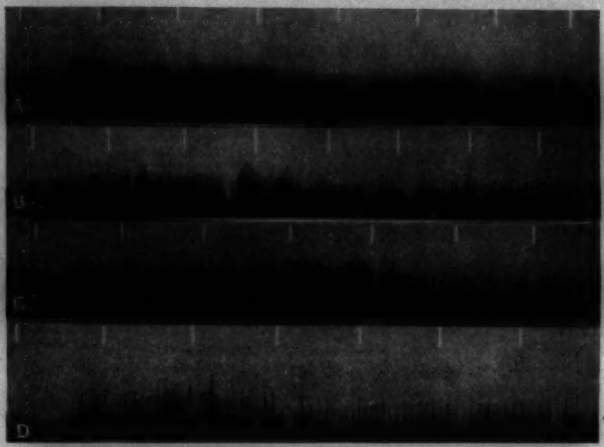

Fig. 1. Electrometer records of proprioceptive impulses from a muscle stretched by 3·2 grm. weight. Each spike represents a single impulse. Nerve was partly cut down. White marks give time in $\frac{1}{4}$ sec. Only the start of each discharge is given. Exp. 18. *A*. Muscle in Ringer. *B*. Muscle in 0·0001 p.c. strychnine for 35 min. *C*. Muscle in 0·001 p.c. for 19 min. *D*. Muscle in 0·01 p.c. for 27 min.: earlier maximum due to slightly more rapid loading.

after 0·01 p.c. strychnine. Another typical experiment is presented graphically in Fig. 2. It will be seen from these records that under the conditions of the experiments strychnine decreased the activity of the end organs, the concentration necessary to produce this reduction usually lying between 0·001 p.c. and 0·01 p.c. Concentrations of 0·001 p.c. or less had no detectable effect as a rule, although in some experiments small decreases were effected with concentrations as low as 0·0001 p.c. It is obvious that this decrease consists not only in a reduction of the maximum impulse frequency but that the frequency throughout the whole discharge is reduced to below the value for normal muscle. In some cases, in fact, there was no trace of the usual long continuing discharge although the initial outburst of impulses still appeared at the beginning of the stretch. And finally, it

is interesting to note that when there was such a long sustained discharge of constant frequency it was not only at a lower frequency level than

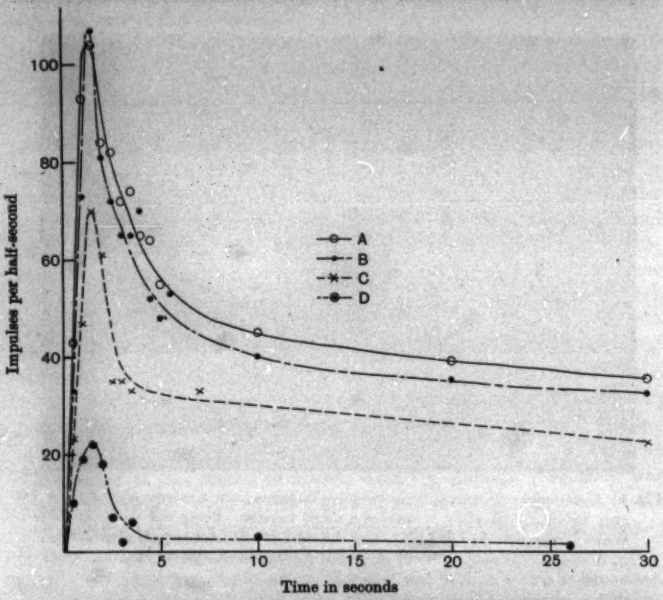

Fig. 2. Number of proprioceptive impulses in successive ½ sec. before and after strychnine. The muscle was stretched every 6 min. with a weight of 4 grm. Exp. 7. *A*. Average of stretches 1, 3, 5 and 7; muscle in Ringer. *B*. Average of stretches 8, 10 and 12; muscle in 0·001 p.c. strychnine. *C*. Average of stretches 13, 15 and 17; muscle in 0·01 p.c. strychnine. *D*. Stretch 18; muscle in 0·1 p.c. strychnine.

before strychnine but this steady level was reached earlier in the course of the stretch.

Although the frequency of impulses coming from an end organ has been shown to increase with the stimulus (5), this rise becomes less and less and finally reaches a maximum value as the stimulus becomes greater. It was therefore conceivable that a possible sensitising action of weak solutions of strychnine had been obscured in these experiments by employing a range of loads such that the end organs were already discharging at their maximum rate, the strychnine consequently being

unable to effect any further increase. Fig. 3 shows that this was not the case inasmuch as it covers the range of loads employed throughout

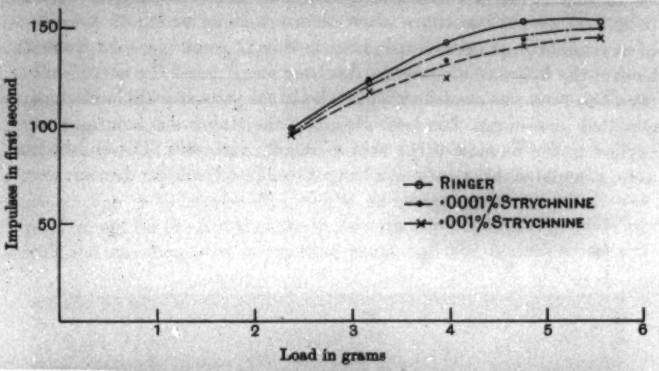

Fig. 3. Curves showing relation between load and frequency of sensory impulses from muscle. Each point is the average of the impulse frequency at corresponding times in two similar stretches. Exp. 17. *A*. Muscle in Ringer. *B*. Muscle in 0·0001 p.c. strychnine. *C*. Muscle in 0·001 p.c. strychnine.

this work. It is typical of a number of experiments in which the variation of frequency with load was determined before and after strychnine.

The evidence cited above shows that sufficiently high concentrations of strychnine decrease the discharge of sensory impulses from a stretched muscle; it does not answer the minor question as to whether the strychnine effects this reduction by reducing the activity of the end organs or by merely abolishing conduction in certain of the intra-muscular sensory fibres. For although care was taken to prevent the solution from coming in direct contact with the nerve, it was obviously not possible to guard against its diffusing into the muscle and there acting on the sensory fibres leading from the end organs. Nor is there any direct way of testing the conductivity of these sensory fibres. A study of the discharge from apparently single end organs, however, shows that the frequency in these regular series of impulses is lower after treatment with the high concentrations of strychnine. This would certainly seem to indicate a decrease in the activity of the end organ.

Strychninisation of pressure endings in skin.

In view of the fact that sensory impulses can be readily recorded in cutaneous nerves the above observations were extended to the effect of strychnine on the pressure endings in skin. A small piece cut from the back of the frog was laid on a hard rubber support and the nerve leading from this area was stretched across the usual non-polarisable electrodes mounted in a moist chamber. Drops of the strychnine solutions were applied to the outside of the skin at regular intervals. The end organs were stimulated by slowly lowering a weighted rubber disc of about

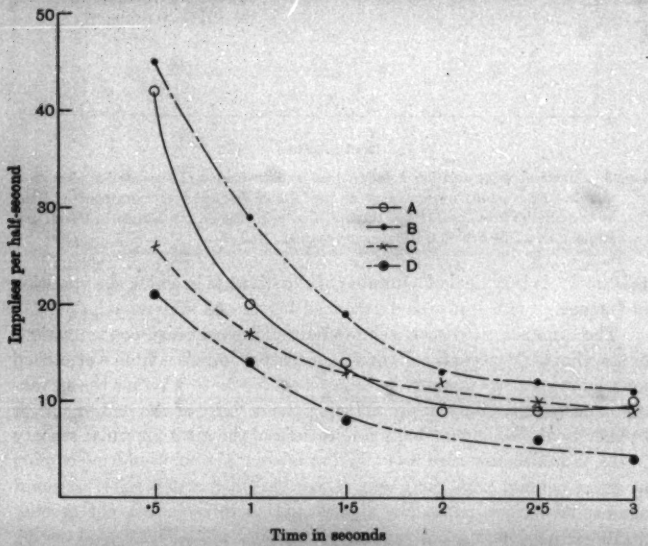

Fig. 4. Frequency of sensory impulses from pressure endings in skin during successive ½ sec. from start of stimulus. Pressure of 0·5 grm. per sq. mm. applied every 5 min. Exp. 20. *A*. Average of stimuli 1, 2 and 3; skin in Ringer. *B*. Average of stimuli 4, 7 and 8; skin in 0·001 p.c. strychnine. *C*. Average of stimuli 11, 12 and 13; skin in 0·01 p.c. strychnine. *D*. Average of stimuli 14 and 16; skin in 0·1 p.c. strychnine.

5 mm. diameter on to the skin, the rate of loading being governed by the dash-pot. The frequency of discharge obtained by thus pressing on the

skin varied greatly from one area to another, probably due to variations in the number of end organs in a given area. Consequently a very slight shift in the position of the stimulating disc had a great effect on the number of impulses produced and made reproducible results more difficult to obtain than in the case of the experiments on muscle.

Bearing in mind the observations on muscle it was rather surprising to find that the majority of these experiments on pressure endings showed a small increase in activity following the application of weak concentrations of the drug (0·0001 p.c. or 0·001 p.c.), although higher concentrations reduced the frequency of impulses as was the case with muscle. Fig. 4 illustrates the results of one experiment which was typical except for the fact that the increase in impulse discharge following strychnine was somewhat larger than usual, and Fig. 5 gives a series of

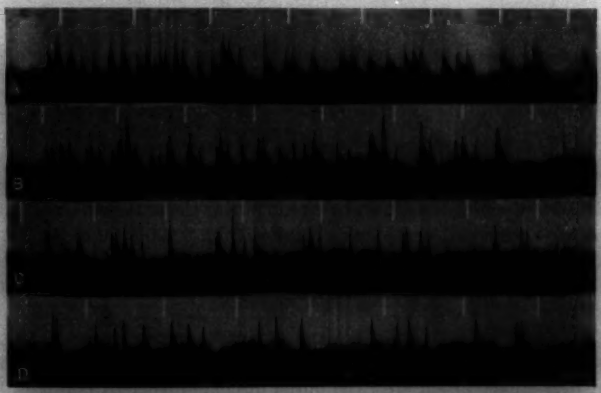

Fig. 5. Electrometer records of sensory impulses (monophasic) from pressure endings in skin. Pressure of 0·5 grm. per sq. mm. *A*. Skin in Ringer. *B*. Skin in 0·001 p.c. strychnine for 12 min. *C*. Skin after 13 min. in 0·01 p.c. strychnine. *D*. Skin in 0·1 p.c. strychnine for 5 min.

electrometer records showing discharges before and after application of the drug. There seems to be no obvious explanation as to the reason why weak concentrations of strychnine act differently upon these two different types of end organs, but it is to be emphasised that with concentrations greater than 0·01 p.c. the effect is much the same and that the weaker concentrations have never produced an increase in activity of the

pressure ending greater than that caused by a 35–40 p.c. increase in stimulus. This is shown by a typical experiment recorded in Fig. 6.

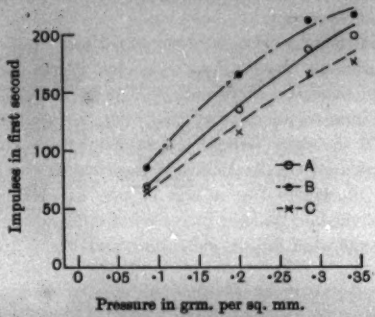

Fig. 6. Curves showing relation between pressure and frequency of sensory impulses from skin. *A*. Skin in Ringer. *B*. Skin after application of 0·0001 p.c. strychnine. *C*. Skin after 0·001 p.c. strychnine.

Summary.

The frequency of sensory impulses coming from a stretched muscle has been determined before and after immersing the muscle in strychnine-Ringer solutions varying in concentration from 0·0001 p.c. to 0·1 p.c.

1. Concentrations as low as 0·001 p.c. usually had little or no effect. In higher concentrations the frequency of impulses throughout the discharge was reduced.

2. Following strychnine the end organs "adapted" sooner than in normal muscle. When treated with the stronger concentrations the impulses stopped soon after the initial discharge whereas normally the discharge continued indefinitely.

3. Weak concentrations of strychnine produced a small increase in the activity of the pressure endings in skin although in higher concentrations it reduced the impulse discharge as it did from the end organs in muscle. There is, therefore, no evidence of strychnine producing a large increase in the activity of either of these two types of end organs as is characteristic of its action on the central nervous system.

4. The method of transecting a large number of the fibres in a nerve trunk has been shown to be a simple means for obtaining impulses from a single sensory end organ or from any desired number.

The writer is exceedingly grateful to Dr E. D. Adrian who suggested the investigation and who has offered every facility for carrying it out.

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THE INFLUENCE OF VITAMIN A DEFICIENCY ON THE ŒSTROUS CYCLE OF THE RAT.

By KATHARINE H. COWARD.

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Evans and Bishop(1) state that "the disturbance of œstrus from fat-soluble vitamin A deficiency is highly characteristic, resembling no other nutritive upset known to us. It consists in the prolongation of the œstrous desquamative change in the vaginal epithelium, the smear consisting chiefly, if not exclusively, of the cornified cells which in normal individuals characterise the actual period of œstrus and ovulation only, but which in the case of animals showing vitamin A deficiency occur throughout the entire period of acute deficiency." They also say that it "may precede all other signs of lack of vitamin A, and furthermore, is shown in conditions where vitamin A is not so low as to cause growth failure or xerophthalmia."

Macy, Outhouse, Long and Graham(2) confirm this and say: "Frequently the first œstrus may be normal, yet the flaky cornified cells indicative of vitamin A deficiency soon become persistent. Usually, however, this characteristic cell is the only type seen from the time of opening of the vaginal orifice until therapeutic methods have been instituted. In all cases where the vaginal orifice has opened beyond the onset of xerophthalmia, the presence of the cornified cells in the epithelial layers of the vagina has been the first and most delicate symptom of vitamin A exhaustion."

Parkes and Drummond(3) examined the vaginal smears of young rats on a vitamin A-free diet for about a month before the final decline and death of the animals. They report more or less normal cycles, some of them somewhat long (*e.g.* nine days), but no case of the occurrence of cornified cells day after day, such as the American workers found.

In applying this test to rats of our own laboratory, several points of interest were encountered which may eventually help in the elucidation of the part played by the ovary or ovarian hormones in the occurrence of œstrus. The subject was approached from two points, (*a*) the young

rat whose stores of vitamin A were depleted early in life, and (*b*) the mature rat whose stores of vitamin A were unknown but certainly not large.

The young rats were pure albinos obtained from a colony which had been fed on a mixed diet proved adequate through five generations. Some of the mature does had been obtained from the same source, used for a vitamin A test and attained maturity. Others were of a black and white strain and had had the same experience since coming to our laboratories. None of the rats had been given any opportunity for mating.

Vitamin A-free diet.

The vitamin A-free diet employed in these experiments was the one in general use in these laboratories (Steenbock and Coward(4)). It consisted of:

Dextrinised rice starch—71 p.c.
"Vitamin-free" casein—15 p.c.
Salt Mixture (Steenbock's 40)—4 p.c.
Dried yeast—8 p.c.
Agar-agar (powdered or shredded)—2 p.c.

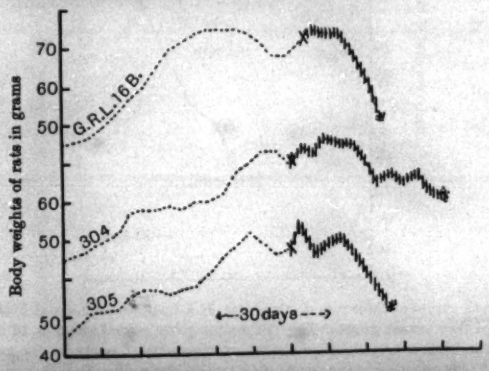

Fig. 1. Growth curves of three rats given a vitamin A-free diet from about four weeks of age until their death. Cornified cells occurred daily in the vaginal smear.

X Opening of the vagina. ✠ Death of the animal.

Each upright line passing through the growth curve signifies the occurrence of nucleated and cornified cells or of cornified cells only (no leucocytes) in the vaginal smear for one day. A shorter upright line, pendant from the growth curve, signifies a mixture of cornified cells and leucocytes.

In order to generate vitamin D in it, it was irradiated in thin layers (200 grm. over a surface of 4 sq. ft.) for 30 min., 2 ft. from a quartz mercury vapour lamp running at 130 volts, 2·5 amperes. It was stirred and respread after the first 15 min. The rats were kept in cages on shavings without screens.

Results.

A. *Young rats.*

Some of our results confirm those of earlier workers. In rats given only the vitamin A-free diet from a weight of 40–50 grm. the vaginas

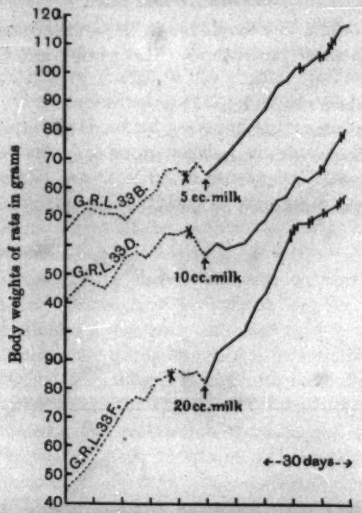

Fig. 2. Growth curves of three rats given a vitamin A-free diet from about four weeks of age until they ceased growth. They were then given graded amounts of fresh milk daily. The vaginas opened before the rats were considered ready for the test, but no cornified cells appeared in the vagina until several weeks after the test was begun.

opened at about 75 grm. weight and 12 weeks of age. The typical heavy, cornified smears occurred every day to the death of the animal. Three records are shown in Fig. 1. The date of opening of the vaginas of these three rats was perhaps slightly previous to the time when, judging from the growth curves, we should have considered the animals ready to be

used for a vitamin A test. This type of response confirms fully the findings of Evans and Bishop, and of Macy and her colleagues.

Wide variations from this result, however, made it appear impossible under all circumstances to rely upon the behaviour of the vagina as a criterion in the diagnosis of vitamin A deficiency.

Fig. 2 shows three records typical of many in these laboratories. In these rats the vagina opened before the rats were considered ready for the test. The first smears consisted of leucocytes only (normally the first smear consists of cornified cells only) and continued to do so. When the rats were steady or declining in weight (seven to ten days later) they were given the particular doses of milk required for the test. They all resumed growth, but only leucocytes appeared in the smears until several weeks later when normal cycles occurred.

Fig. 3 gives the records of four rats whose vaginas did not open until

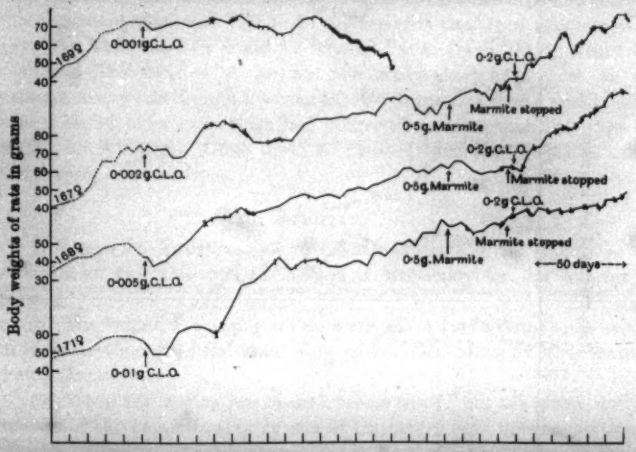

Fig. 3. Growth curves of four rats given a vitamin A-free diet from about four weeks of age until they ceased growth. They were then given graded doses of cod liver oil which made them resume growth. The vaginas did not open until several weeks after the test had been begun and normal cycles were established only when the doses of cod liver oil were made big enough to promote growth to maturity. The vagina of rat 167 had opened before observations were begun.

some time after the vitamin A test had been started. Obviously, the contents of the vagina could not be used in these rats in the diagnosis

of vitamin A deficiency. All four animals showed more or less feeble attempts at cycles, but these ceased and were followed by long periods of leucocytes only. The rat having the lowest dose of cod liver oil declined in weight and eventually died, but for the last five weeks of its life had cornified cells daily. The addition of extra vitamin B in the form of a daily dose of 0·5 grm. marmite for 32 days to the diet of the other three rats made no difference to the rate of growth or to the contents of the vagina. The marmite was therefore discontinued and a larger dose of cod liver oil, 0·2 grm. daily, was given. This made the two lighter rats (113 and 94 grm. respectively) resume rapid growth. The heaviest one, 126 grm., which was also the one which had received the largest

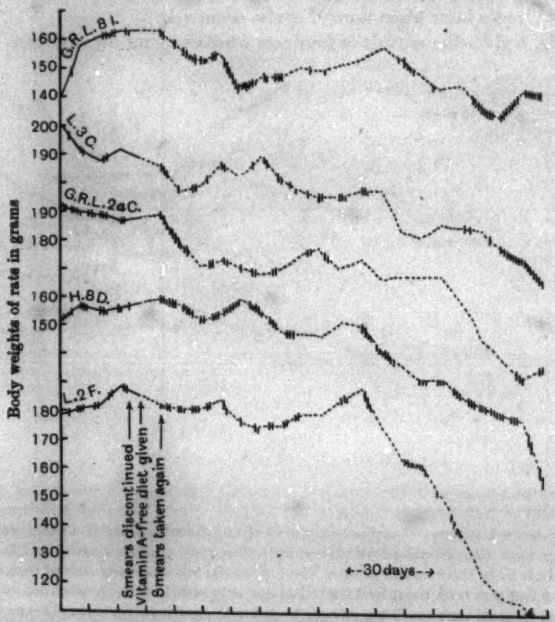

Fig. 4. Growth curves of mature rats given a mixed diet for several weeks, then a vitamin A-free diet. Cycles were fairly normal at first, then followed long periods in which leucocytes only occurred in the vagina, and later followed periods in which cornified cells only occurred almost daily.

dose of cod liver oil before (0·01 grm.), continued its slow rate of growth. In all three rats perfectly normal regular cycles were established.

B. *Older rats.*

Rats which had already been used for vitamin A testing and had grown to maturity were examined daily for 24 days to see whether cycles were recurring normally. As it was evident that they were, no smears were taken for five days. The rats were then given only the vitamin A-free diet, and after seven days of this smears were taken daily for about 130 days. Fig. 4 shows five typical results. Cycles occurred more or less normally for some time, though in three animals the œstrous period tended to be long and the diœstrous short. Each animal then had a long period in which only leucocytes occurred in the smears, and this in certain animals was followed by a period in which only cornified cells occurred. The rats which had not already died under this treatment were then killed and their ovaries examined microscopically. No abnormalities could be found. Parkes and Drummond (3) examined the ovaries of certain rats which had been kept on a vitamin A-free diet from a low weight, 40–50 grm., for many weeks, and also those of older rats which had recovered on a normal diet after a period on a vitamin A-free diet. In none of them did they find any histological abnormalities.

Summary.

It appears to be impossible to use the contents of the vagina as a criterion in the diagnosis of vitamin A deficiency for the following reasons.

(*a*) The vagina of a young rat on a vitamin A-free diet may not open until some time after the usual signs of vitamin deficiency have manifested themselves.

(*b*) When the vagina has opened before these signs are given, daily smears of the contents may consist of leucocytes only until some time after the curative dose of vitamin A has been given, and then normal cycles occur.

(*c*) The smears may, however, consist of cornified cells only, and if no dose of vitamin A is given the smears may consist of cornified cells up to the death of the animal.

When mature, unmated rats are given a vitamin A-free diet they have normal cycles for some time. Their smears then come to consist only of leucocytes and later may consist only of cornified cells.

The writer would express her thanks to Miss Marjorie R. Cambden for much help in the taking of the smears in this experiment.

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THE EFFECT ON THE RESPONSES OF THE SOLEUS MUSCLE OF AN ALCOHOL BLOCK ON THE SCIATIC NERVE.

By E. G. T. LIDDELL and J. M. D. OLMSTED.

(*From the Laboratory of Physiology, Oxford.*)

If alcohol is applied to a nerve, and the times observed at which various reflex responses mediated by the nerve are impaired, it is possible to gain information, as Forbes and Olmsted have shown, regarding the mode of production of the various forms of reflex activity (6).

Method.

The muscle chosen for these experiments was the "red" soleus muscle, and the forms of activity investigated were the stretch reflex, the tendon jerk, crossed extension reflex, and their inhibition. For purposes of comparison the response to direct stimulation of the motor nerve was investigated as well.

The soleus of the left side was used for observation. The pudic, hamstring, obturator and femoral nerves were severed on both sides of the body, as well as the iliopsoas muscle. On the right side the sciatic was divided just above the knee and the central end enclosed in shielded electrodes for stimulation of the crossed extension reflex. On the left side all the branches of the popliteal except that going to the soleus and corresponding head of gastrocnemius were severed at the knee. In some cases the left peroneal was severed at the knee and the central end enclosed in shielded electrodes for inhibition; in others the branches of the peroneal going to the tibialis anticus muscle were used as an afferent and were left intact, the main division of the nerve being severed just peripheral to these branches. In the latter cases the tendon of tibialis anticus was separated (with a small piece of bone) and cut free from its attachment to the foot so that it could be stretched by hand and used as an inhibitory agent. Electrodes with a slotted shield were placed on the left sciatic far up in the hip for direct motor-nerve stimulation. Fig. 1 shows diagrammatically the general arrangement.

The animals (cats) were decerebrated under deep anæsthesia at the intercollicular level and allowed to recover.

To ensure the non-interference of the gastrocnemius, the tendon of soleus was carefully dissected free from the mass of tendons composing

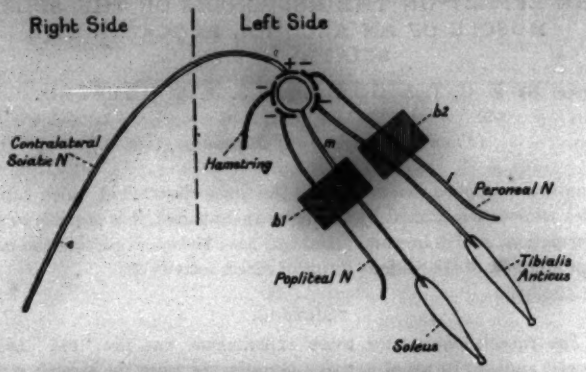

Fig. 1. *m*, region of popliteal nerve, stimulated for motor response in soleus.
c, region of contralateral sciatic nerve stimulated for crossed extensor in soleus.
i, region of peroneal nerve stimulated for inhibition in soleus.
b^1, position of alcohol block to popliteal nerve only.
b^2, position of same block to peroneal nerve as well.
The afferent paths from soleus and the efferent path to tibialis anticus are not shown.

the Tendo Achillis, and all except that of soleus were severed. The piece of calcaneum left to facilitate attachment of the tendon to the isometric shadow myograph could therefore be moved only through the action of the soleus muscle. Drills through the tibia at ankle and knee, and also a pelvic clamp afforded rigid fixation to the warm table on which the preparation lay. The optical system was so adjusted that when the shadow of the lever moved through a distance of 1 mm. a change in tension of 30 grm. was indicated. The same table with falling top was used to elicit the stretch as had been used on former occasions (9). The distance of fall was 0·8 cm. and the time of fall 2 sec.

For electrical stimulation Sherrington torsion wire keys delivering 48 and 49 break-shocks per sec. were used in combination with coreless coils having 2 volts in the primary circuit.

Different percentages of ethyl alcohol in Ringer (12–16 p.c.) were used (6). The choice of the strength of alcohol depended upon the nature of the experiment, *e.g.* 13 p.c. will often extinguish stretch reflex and

jerk, and not affect crossed extension or the motor response. The alcohol was applied to the left popliteal nerve in the following manner: a swab of cotton wool approximately 1 cm. wide and 2 cm. long, soaked in the solution, was wrapped about the nerve midway between the knee and hip, the peroneal nerve being out of contact with the swab (Fig. 1, b^1). The wound was then closed and the skin edges held together by a clip. When the peroneal nerve was narcotised as well, the one swab was wrapped round both nerves (Fig. 1, $b^1 + b^2$). This procedure evidently caused little or no injury to the nerves since complete recovery of all reflexes occurred within some five minutes after removal of the swab.

Argument.

It is probable that the maximum rate at which impulses can succeed each other along a given nerve is limited by the length of the absolute refractory phase of that nerve. If a region of block is established at any point along a nerve by the application of narcotics or cold, impulses of a sufficiently high frequency will suffer a reduction on meeting the block if the refractory phase has been made longer by narcosis than the interval which exists between any two or more impulses (10). In other words, the least interval between any two impulses passing that region is increased and the maximum rate at which a series of impulses can pass the region is diminished.

Comments on the myographic method of analysing reflex actions.

In assessing the relative frequency of discharge of the impulses, as we have done, by the size of the myographic response, consideration has to be paid to several points.

(1) *The narcotisation of the nerve trunk.* The strengths of alcohol which we employed for producing a block usually abolished entirely the crossed extension reflex within 10 min. of the application while the direct motor nerve response was very considerably diminished during that time. After removal of the block, recovery occurred rapidly and was often complete, so far as the crossed extensor response was concerned, in a period of time less than half that required for the production of the block, and for the motor response often one-quarter of the time. The nerve to soleus is a small nerve lying superficially on the main popliteal trunk and is traceably distinct from it for some distance. It was presumed that the alcohol penetrated by diffusion into the tissue-spaces of the nerve, affecting the outer fibres first. It might be objected that since the blood supply of the

nerve was intact, the degree of block in differently situated fibres was unequal. We have no direct evidence that this was not the case. But the results of different experiments were never greatly discrepant, and for our purposes, in any one experiment, and with a series of observations made in sequence with only a few seconds between each observation, there was no reason to suppose that any very sudden change in state of any fibre supervened between each observation, but only between each series of observations.

(2) *Difference between afferent and efferent fibres in respect of susceptibility to blocking.* We were not able to detect any difference of this kind. Impulses generated by an electrical stimulus (48 break-shocks per sec.) in an afferent nerve trunk and passed through an alcohol block still produced general reflexes in the cat—though weakened—at a time when the reflexes used in our tests were abolished. At that juncture the motor nerve (to which was applied the same block) still responded to stimulation, although less strongly than at the beginning. In other words, impulses generated by similar rates of electrical stimulation in afferent and efferent fibres seemed to suffer extinction at the block *pari passu*.

(3) *Response of nerve centres to summed stimuli.* Summation of effect at the final motor neurones, as judged myographically, may be brought about (*a*) by employing more afferent fibres whereby (i) more motor neurones are excited in a supraliminal degree to discharge impulses, (ii) more neurones, which previously were on the fringe of an excitation and undergoing subliminal excitation without discharging impulses (4) now respond to further excitation, in itself subliminal, by discharging impulses since the two subliminal excitatory processes would have summated to produce a supraliminal degree of excitation; (*b*) by the "strength duration" factor of impulses at nerve centres, *e.g.* (i) by repetitive subliminal excitation at a fixed number of neurones mounting up in a long period of time to a supraliminal value, thereby producing a discharge of impulses (*e.g.* scratch reflex (11)); (ii) by close synchronism of the volleys in a few afferent fibres whereby an excitatory state (*E*) (13) is evolved within a brief space of time to an effective threshold at the reflex centre, thereby producing a discharge of impulses (tendon jerk or "pluck").

These types of summation of central effect, either separately or both together, may be modified by an alcohol block on the afferent nerve, since in any given trunk some fibres which are partially blocked may transmit impulses at a diminished rate and possibly of diminished intensity (see pp. 42, 43), while other fibres may be completely blocked

and transmit no impulses. These problems of summation are discussed further in Fulton's monograph (7).

Augmentation by the crossed extension reflex of the stretch reflex and tendon jerk.

In practically all cases where the stretch reflex was well marked, it was found that crossed extension caused a marked increase in the tension developed in succeeding stretches. Thus the first stretch some 12–15 sec. after crossed extension often developed a tension three times that originally shown. The effect diminished gradually within 2 min. as is shown in the upper curve of Fig. 2, but did not always disappear com-

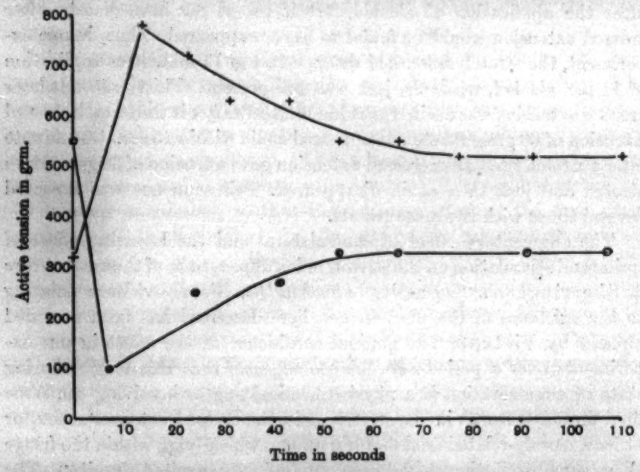

Fig. 2. Upper curve: enhancement of stretch reflex following 3 sec. stimulation of contralateral sciatic nerve.

Lower curve: depression of stretch reflex following 2½ sec. stimulation of ipsilateral peroneal nerve.

pletely as shown by the fact that although succeeding stretch reflexes finally exhibited a constant degree of tension this was often decidedly greater than before crossed extension. The average time for the stretch reflex to return to a constant value (5 trials) in this preparation was 75 sec. This same average time (75 sec.) was found for 4 trials in another

preparation. In two others there was only temporary augmentation of the stretch reflex, the return to normal occurring in 105 sec. (3 trials) in one case, and in 50 sec. (2 trials) in the other. In the latter case there was an immediate complete inhibition of the stretch reflex after crossed extension; augmentation then followed.

The contrary effect was seen after stimulation of the ipsilateral peroneal nerve (lower curve of Fig. 2). Here the effect again diminished gradually over a period of 50 sec., but persisted since the tension of succeeding constant stretches was decidedly lower than before stimulation of the peroneal nerve.

The bearing of this phenomenon on our experiments is obvious. A normal stretch might be definitely extinguished at a certain moment after the application of alcohol, yet if tested for immediately after crossed extension would be found to have reappeared. Thus, in one experiment, the stretch reflex had disappeared in 13 min. after application of 14 p.c. alcohol, while the jerk was still present. Thirty seconds later crossed extension was elicited, and immediately after it the stretch showed a tension of 60 grm. Stretch disappeared again within 45 sec. One minute later a stretch tried after crossed extension gave a tension of 30 grm. Both stretch and jerk then again disappeared. This sequence was repeated several times with the same results.

The augmentary effect of contralateral and the lessening effect of ipsilateral stimulation on the stretch reflex appear to be of the same nature as Sherrington's "successive induction" (12). Some evidence pointing to the existence of the phenomenon here described has been recorded already by Viets (14). The gradual subsidence of the effect in our experiments over a period of a minute suggests that this long enduring state of augmentation is a physicochemical one (13) involving the same final motor neurones in the stretch as in the crossed extensor reflex, or at least closely-related final motor neurones which lying within the fringe of crossed excitation have been subliminally excited by it (4). The phenomenon is in agreement with previous suggestions that have been made regarding the long duration of excitation and inhibition at nerve centres (2), (7).

Stretch reflex and jerk in relation to narcotisation.

There can be no doubt that of all the reflexes investigated in these experiments the stretch is the most easily extinguished by the alcohol block. Indeed, rough manipulation or exposure of the popliteal nerve rapidly abolishes it without further agency. With 11 p.c. alcohol (Fig. 1,

b^1) it was possible to cause the stretch reflex to disappear while the jerk remained. Two experiments are of especial interest. In one, with 13 p.c. alcohol, the stretch reflex disappeared 5 min. after the continued application of alcohol, followed 2 min. later by the jerk; but if elicited immediately after crossed stimulation (augmentation) the jerk could be caused to appear at any time for over a period of 24 min., while a stretch reflex was not so obtained. In the other, with 14 p.c. alcohol, stretch reflex and jerk diminished so rapidly that it was not possible to distinguish between their relative rates of subsidence and they disappeared apparently together in 2 min. 20 sec., but reappeared through augmentation after crossed extension for a further 14 min. During the succeeding 20 min. the jerk, but not the stretch reflex, could be elicited through augmentation, the alcohol during this period being without effect on crossed extension.

The stretch reflex and jerk are considered to involve the same mechanism, the jerk being a "kinetic" or "phasic" stretch reflex. Why, therefore, should the stretch reflex disappear first and recover last? It is well established that in the stretch reflex there is a maintained repetitive fire of centripetal impulses (1). With a lengthening of the least interval as a consequence of narcotisation the impulses, if in rapid sequence, will be reduced in number, so that their central effect at the final motor neurones will be diminished. In the jerk, on the contrary, there is one synchronous or nearly synchronous afferent volley. Since by this hypothesis the volley of impulses in the jerk is more synchronous than in the stretch reflex, the massed effect of those afferent impulses at the spinal centres is more productive of effective excitation than is the arrival of afferent impulses in the stretch reflex which are widely dispersed in time (11). Owing to the lengthening of the least interval in the nerve trunk, the impulses in the stretch reflex, previously dispersed in time, are now also diminished in number to a degree which fails to produce an excitatory state at the final motor neurones. So the stretch reflex wanes. In the jerk, however, as elicited in the (ordinarily) quiescent muscle, the muscle organs are under a slighter tension, and therefore discharging less frequently (1). On the grounds of probability a tap delivered to the tendon organs will cause a discharge of impulses, of which the first of each has a relatively greater chance of finding its nerve fibre in a non-refractory state. A synchronous volley of single impulses in this way reaches the final motor neurones and their synchronicity allows them to sum their effect there and to produce supraliminal excitation and discharge of efferent volleys, resulting in the "jerk". Later, in

narcotisation, of course, there will be a complete extinction of all impulses, however timed.

Crossed extension and motor response.

The stretch reflex disappears before crossed extension, and the latter in turn disappears before the motor response. In fact it is difficult to cause the motor response to disappear completely with the strengths of alcohol generally used. The difficulty might be due to "escape of current" past the block. The stimuli, however, were weak, for the secondary coil of the Berne inductorium (2 volts in primary and coreless) was placed at 2750 on the Kronecker scale. At this strength the shocks were just perceptible to the tongue and the coils were just overlapping. The secondary coil had to be at 11,500 on the Kronecker scale to stimulate the muscle maximally. The narcotising swab was distant 2 cm. from the electrodes, or more. In one experiment 13 p.c. alcohol caused the crossed extension to drop to less than 10 p.c. of its original value while the motor response was still at its original value, the rate of stimulation of the nerves being 48 and 49 break-shocks a second as usual. Again, with 15 p.c. alcohol, crossed extension dropped to 80 p.c. in 9 min., the motor response at that time showing no effect; at 14 min. crossed extension was now 15 p.c., the motor response 90 p.c.; at 17 min. crossed extension 4 p.c., motor response still 90 p.c., at 19 min. crossed extension had virtually disappeared, while the motor response was 80 p.c.

Such figures as these support the inference previously made by Forbes and Olmsted(6) that the rate of central discharge from the motor neurones in the crossed extensor reflex is more frequent than the rate of direct motor nerve excitation—here 48 break-shocks per sec.

Since the popliteal nerve contains sensory fibres from the soleus as well as motor fibres to it, sensory impulses from the muscle itself might conceivably be responsible for the above results. Therefore it was thought advisable to repeat the observation on a preparation with the left soleus deafferented. It was readily seen that in the deafferented muscle crossed extension disappeared some minutes before the motor response.

These results show that in general the sequence of disappearance of responses after establishing an alcohol block is first the stretch, second the jerk[1], third the crossed extension reflex, fourth the motor response. This is shown in the graph (Fig. 3).

[1] On one occasion only, from among ten preparations, did the jerk persist after the crossed extension reflex.

Recovery from alcohol.

The reverse sequence was noted during recovery from alcohol. The following are examples of extreme differences observed on different

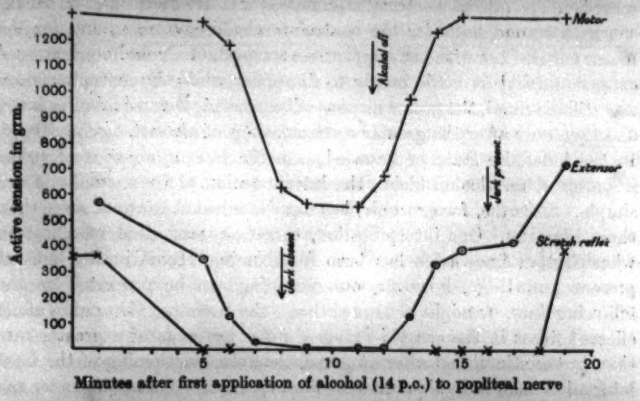

Fig. 3. Ordinates indicate active tension in grm. Abscissæ time in minutes after application of alcohol to the popliteal nerve (b^1 of Fig. 1). Upper curve: motor response; middle curve: crossed extension; lower curve: stretch reflex. The time of disappearance and of reappearance of the jerk is indicated, as well as the time of removal of the alcohol.

occasions: (i) the motor response recovered completely while crossed extension was still extinguished—the latter recovered 3 min. later; (ii) crossed extension returned to full original value while jerk was still absent; (iii) jerk returned 6 min. earlier than stretch. In general, however, the motor response had only partially recovered by the time crossed extension made its first appearance. The jerk also usually appeared before complete recovery of crossed extension. The stretch reflex invariably returned late.

Forbes and Olmsted(6) suggested on the basis of experiments with the alcohol block method that the majority of impulses in the motor nerve during a crossed extension reflex are subnormal, as judged by the length of least interval for two impulses required at that depth of narcotisation to abolish the crossed extensor reflex. The rate of discharge of the impulses was such that it was diminished by a depth of narcosis which impaired one impulse following another within 3σ. This meant that the maximum rate of discharge of impulses must have been about

330 per sec.[1] The muscles used in the experiments of Forbes and Olmsted were the extensors of the lower limb, comprising both "pale" and "red" muscles of the gastrocnemius-soleus complex. In the experiments described in this paper the action of a "red" muscle alone is recorded. The rate of electrical stimulation was not more than 50 break-shocks a second both for the contralateral afferent nerve and for the motor nerve. Yet with the appropriate strength of alcohol block crossed extension could be made nearly to disappear while the motor response was still maximal, the motor response disappearing several minutes later, and then only after the greater concentrations of alcohol, *e.g.* 15–16 p.c. In the indefinite state of knowledge on the fate of a series of nerve impulses at an alcohol block, the interpretation of these results is not simple. Kato(10), for example, has not described the fate of more than three impulses. Our interpretation, therefore, may need modification when further knowledge has been forthcoming. Even in the light of present knowledge, however, our reasoning can be presented on the following lines. It might be argued that if the discharge of impulses along efferent fibres in the crossed extensor reflex proceeds at a greater rate than in the stimulated afferent fibres, then the lengthening of the least interval in each individual efferent fibre by the alcohol accounts for the diminution in the frequency of impulses in a series and a fall in tension of the reflex. But the number of normal impulses a second received by a muscle makes little difference to the tension developed until the rate is very much lower than 50 a second(5), especially for a slow muscle like soleus. Here it is supposed that the rate of reflex impulses is normally very much higher than 50 a second, while the motor nerve itself is receiving directly 48 impulses a second and is but slightly impaired. This interpretation, then, fails as it stands and is further weakened by its inability to explain the fate of the first impulse of a series which would be full-sized and presumably show some effect, *e.g.* "opening twitch"(9*a*) in both reflex and motor response, even though in the former fewer fibres are activated. If, however, the conception used by Forbes and Olmsted of the "subnormal impulse" be employed here, it may then be hypothesised that each impulse in a reflex series is smaller than each impulse in a motor series and will be more liable to extinction in the block. To deal with the non-effectiveness of the first impulse of a subnormal series the conception needs enlargement to include the possibility of peripheral summation at nerve endings in muscle or at the region of

[1] Observations recently made by Denny-Brown (Thesis for D.Phil. Oxford, 1928) definitely establish a much lower rate of central discharge.

block in the nerve, so that the muscle fibre responds only after receiving two or three impulses ("addition latente"), for which event evidence is not lacking (3, 3a-d). By acceptance of these combined hypotheses, the lack of effect from the first impulse may be explained (absence of peripheral effect) as well as the diminution in frequency of the series (subnormal impulses) for the stage of narcosis at which the crossed response is just banished and the motor response is still strong. Later, when the narcotisation has progressed to a degree of depth at which the motor response alone is left and that is severely impaired, one would expect that if the first impulse, after passing the block and recovering its intensity, is the only normal impulse of the series, an "opening twitch" would be observed in the muscle. But we never did observe an opening twitch at this or any other stage of narcosis and were driven to conclude that even the first impulse of the motor series was not effective. There must, therefore, have been established a state in the nerve at which the first impulse (or impulses) failed to pass while subsequent ones did pass the block and produce a contraction without an opening twitch. Records produced under such conditions have been published by Goldscheider (8), his Fig. 17). Unfortunately at present we have no photographic records of our responses for comparison and further experiments are needed. To observation, however, our motor responses were of the gradually ascending type figured by Goldscheider. It appears then that in our experiments and those of Goldscheider there was a summation of effect from the impulses occurring within the blocked region of nerve. Adrian and Lucas (3d) from their observations on the summation of propagated disturbances concluded that the nerve endings are "not to be credited with any specific property essential to the production of this type of summation. Their part in the phenomenon is apparently no more than the offering of such imperfect conduction that single propagated disturbances are unable to break through—the only special condition of a tissue necessary for the production of such a summation is the presence of a region impassable to a single propagated disturbance." Our reasoning then leads likewise to the belief that summation of the propagated disturbance ("addition latente") is a property of narcotised nerve fibres. If that assumption be permitted, it is not necessary in the preceding argument to rely on the supposition that nerve endings show the same behaviour in the face of subnormal impulses. That the region of narcotised nerve alone shows "addition latente" is an adequate assumption, although the existence of "addition latente" in the nerve ending would provide an auxiliary mechanism for the summation of propagated disturbances. Moreover,

this latter postulation of summation at nerve endings runs counter to the observations of the Lapicques[84] on the iso-chronicity of nerve fibre and muscle fibre, although their observations were made with cut nerve which has a higher chronaxie than uncut[85]. Therefore on the grounds of being imperfectly established and here superfluous, the conception can be discarded from our argument.

Another experimental factor to be considered is the selective action of alcohol on the different fibres in the motor nerve, some being much more resistant than others. It is to be presumed that those impulses in crossed extension which survive in spite of the alcohol are almost full-sized and occur in the more resistant fibres. The afferent impulses of the stretch reflex and jerk, on the contrary, are initiated by the action of the receptor organs in the muscle itself. If, as suggested above, the impulses set up by the jerk are almost synchronous, it might be thought that the jerk would outlast crossed extension. This, indeed, did happen once, and might *ex hypothesi* be expected frequently to occur. As a general rule, however, the great supraliminal excitation ("central drive") of a large number of final motor units in the crossed extensor response exceeds in numbers and in force any reflex response from a momentary tendon tap, for even if from each final motor unit involved in the crossed extensor response the first impulse alone is able to pass the block at that stage of narcotisation, the number of muscle units so operated is large enough to record a marked degree of muscular contraction in the myograph. In the jerk, on the other hand, the afferent fibres are far fewer than in the crossed nerve and are suffering from the block, which the crossed nerve is not. For these two reasons the "central drive" from the tendon tap is, in comparison, a paltry affair, and in the large majority of our cases fails before the crossed nerve to excite a reflex from the spinal centres.

Inhibition.

A preliminary experiment on a preparation in which the soleus was deafferented by spinal root section showed that when the ipsilateral hamstring nerves (un-narcotised) were stimulated, inhibition of crossed extension was prompt and complete as long as this reflex was present after establishing the alcohol block on the popliteal nerve. Thus in one case the tension developed before application of alcohol was 630 grm. This was inhibited to zero upon inter-current stimulation of the hamstring nerves. After application of alcohol to the popliteal nerve (Fig. 1, b^1) and when the tension of crossed extension had been reduced to 15 grm.

(the lowest level of tension reached), it could still be unmistakably inhibited. This is to be expected, if the inhibitory effect is central and not peripheral, as is undoubtedly the case.

Ten experiments were performed, in which the ipsilateral peroneal nerve was prepared for stimulation with shielded electrodes at the knee (Fig. 1, *i*). At the hip shielded electrodes were placed on the sciatic nerve for motor responses (Fig. 1, *m*). The alcohol block was applied to the whole sciatic nerve between the two pairs of electrodes and some 2 cm. from either (Fig. 1, $b^1 + b^2$). With 14 p.c. alcohol the stretch reflex and jerk disappeared in due order, but reappeared through augmentation and could for a time be inhibited. Later after the stretch reflex had quite disappeared the augmented jerk could be inhibited. Crossed extension at this time was still maximal, 660 grm., and could be completely inhibited. A second trial gave similar results. With 15 p.c. alcohol in the same preparation both crossed extension and inhibition could often be made to disappear. Thus in one trial crossed extension remained and inhibition disappeared. In two others the reverse was found[1]. Again, in two trials crossed extension disappeared first, in two others inhibition first. On recovery from alcohol, crossed extension appeared before inhibition in three trials, and the two appeared simultaneously in a fourth. It is evident from these equivocal results that the effect on the afferent fibres in the peroneal and the motor fibres in the popliteal are affected in not widely divergent degrees by the alcohol block. The results may also be taken as evidence of the simultaneity of narcotic effect in penetrating the nerve trunk. Since the nerve to soleus is superficial and almost detached, it might happen with slow penetration of the drug, the motor fibres would be completely narcotised at a time when the afferent fibres in the peroneal trunk causing reflex movement in the tail and fore-limbs were unaffected. That full narcotisation occurred at about the same time in both sets of fibres showed that narcotisation must have been approximately of equal depth throughout the region of the block in the nerve trunk. The results also refute a simple and obvious interpretation which Forbes and Olmsted had to face, that of "unequal narcotisation of the component fibres of the nerve." For if that did occur, it might then be argued that as the motor response involves numerically more units than any of the reflexes, as is clear from our Fig. 3, the narcotic will need to be applied for a longer time for the motor

[1] When there was no background of crossed extension reflex against which to test the inhibition the evidence for the transmission of impulses along the inhibitory nerve was presumed from reflex movements in the fore-limbs, trunk and tail.

in order to block the greater number of fibres engaged. That, however, does not appear to be arguable from our results.

When with the block again applied to the whole sciatic nerve (Fig. 1, $b^1 + b^2$) the inhibiting stimulus was a "pluck" (12 oz.) given by hand on the tendon of the tibialis anticus muscle, instead of break-shocks at the rate of 49 per sec. on the peroneal nerve, the results were slightly different. In four trials on one preparation 11 p.c. alcohol caused inhibition against crossed extension to fail before crossed extension while the stretch reflex was inhibited as long as it persisted. This strength of alcohol had indeed no effect on the tension developed in crossed extension, and when the pull on tibialis anticus had reached the stage of failing to inhibit this reflex, intercurrent stimulation of the peroneal nerve peripheral to the alcohol block (Fig. 1, *i*) at the usual rate immediately caused the tension to drop from 900 grm. to zero. After removal of the alcohol, a "pluck" on the tibialis anticus began to show its inhibitory effect on crossed extension before the stretch reflex returned, and at the first appearance of a stretch reflex the latter was inhibited by a "pluck" on tibialis. This may be interpreted to mean, in accordance with the hypothesis, that the impulses set up as a consequence of the continuous action of the proprioceptive sense organs during the stretch reflex are more asynchronous and possibly smaller than these produced by a brief "pluck" on the tibialis—an argument akin to that used for the tendon jerk (1). The failure of the "pluck" as an inhibitory agent at a time when electrical stimulation of an inhibitory nerve had an apparently undiminished effect may be explained on the supposition that the "pluck" excites a small number of afferent fibres. These, owing to the block, transmit never more than one impulse to a small number of central loci, producing there only a weak tendency to an inhibitory state (formation of *I* substance) (13). The continued electrical stimulation of an inhibitory nerve on the other hand excites impulses in many, if not all, available fibres, which, so long as the "least interval" of the nerve is not infinite, pass in more or less number to the central loci and there summate temporally to produce a strong state of inhibition. In another preparation 13 p.c. alcohol caused the stretch to disappear, inhibition being possible as long as the stretch reflex was present, while the "pluck" on tibialis was still able to inhibit crossed extension from 330 grm. to 90 grm. When crossed extension had disappeared, direct stimulation of the peroneal nerve caused the tail of the preparation to move, showing that some afferent fibres were still able to conduct. In another trial the stretch reflex could be inhibited by the "pluck" as long as it was present, but crossed extension persisted at

about one-fifth of its original value and could still be almost completely inhibited by a pull on tibialis.

Summary.

1. After elicitation of the crossed extension reflex in the decerebrate cat, the tension developed by the soleus in the stretch reflex is often augmented threefold. After stimulation of the ipsilateral peroneal nerve it is often diminished (Fig. 2). In both cases the effect is long lasting, more than 90 sec. It seems probable that the same final motor neurones are involved in both reflexes.

2. During narcotisation of the popliteal nerve responses of the soleus disappear in the following order: first, stretch reflex; second, jerk; third, crossed extension; fourth, motor response.

Reasons for this order of disappearance are put forward and discussed at length in the text of the paper.

3. Alcohol influences afferent fibres in the peroneal nerve and motor fibres in the popliteal to a similar degree.

4. During progressive narcotisation of the sciatic nerve, including both afferent and efferent fibres, if any inhibitory impulses reach the "final motor neurone" they are effective against the stretch reflex. When the stretch reflex has been banished by the alcohol block, a pull ("pluck") on tibialis anticus may still inhibit crossed extension, and when the pull on tibialis anticus loses its effect, stimulation at 49 per sec. on peroneal nerve may still inhibit the crossed extension reflex.

The presentation of our theme owes much to the suggestive criticism of Dr D. E. Denny-Brown.

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THE DETERMINATION OF THE CARBON DIOXIDE CONTENT OF THE MIXED VENOUS BLOOD.

Part I. The effect of oxygenation and the critical oxygen tension.

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The method of Henderson and Prince(9) for determining the CO_2 partial pressure in the mixed venous blood by a series of repeated rebreathings from a bag containing a suitable gas mixture has been modified in various details by subsequent workers (*e.g.* Meakins and Davies; Field, Bock, etc.). In any such method the maintenance of the oxygen partial pressure in the rebreathing bag at a level that ensures full oxygenation of the blood entering the lungs when the gas is inhaled at each rebreathing is a condition of outstanding importance, if the CO_2 partial pressure is to be applied to the dissociation curve of fully oxygenated blood for the purpose of ascertaining the CO_2 content. If the venous blood is not fully oxygenated, the CO_2 partial pressure will be lower and the CO_2 content read from the dissociation curve will be less than the actual value. Such methods are based on the observations of Douglas and Haldane(4) who verified *in vivo* the effect of oxygenation on the CO_2 combining capacity of the blood which was established *in vitro* by Christiansen, Douglas and Haldane(2). Douglas and Haldane give data from which it can be shown that the same value for the arterio-venous CO_2 difference in volumes per 100 c.c. blood is obtained whether it is computed on the basis of true venous or oxygenated venous bloods.

The errors caused in the values of CO_2 partial pressure and content caused by insufficient oxygenation may not be large if the unsaturation is not great; but when these data are used in the calculation of the minute volume of the heart's output the influence of such errors becomes greatly enhanced.

It is of interest to see what effect an error of known magnitude in the determinations required for the measurement of the minute volume by the Fick principle will have on the final result.

The variables to be considered are:

(1) The CO_2 pressure difference between arterial and venous bloods, the venous blood being supposed to be fully oxygenated without change of its contained CO_2.

(2) The arterio-venous CO_2 difference measured in volumes per 100 c.c. blood.

(3) The CO_2 output per minute.

(4) The minute volume.

If p = the arterio-venous CO_2 pressure difference in mm.,
v = the arterio-venous difference in volumes p.c.,
c = the CO_2 output per minute in c.c.,
m = the minute volume of the heart in c.c.,
then, from Fick's principle,

$$mv = 100c \qquad \text{.....(1).}$$

Now there are to be considered absolute and relative errors. The absolute errors are directly proportional to the magnitude of the minute volume. Relative errors are, however, more complex and must be considered further.

Relative errors in the determination of the arterio-venous CO_2 pressure difference.

The first is that in reading the volumes p.c. corresponding to a given CO_2 pressure. This can be neglected if the CO_2 dissociation curve of the subject be accurately known. The well-known curve determined by Christiansen, Douglas and Haldane(2) shows that between 38 mm. and 60 mm. of CO_2 the relation between volumes p.c. and CO_2 pressure is practically a straight line. This line approximates closely to the equation

$$y = \frac{2}{5}(x + 100) \qquad \text{.....(2).}$$

When considering differences only, this can simply be written

$$5\Delta y = 2\Delta x,$$

or, using the special symbols given above,

$$5v = 2p \qquad \text{.....(3).}$$

This gives the relation that, between the limits specified, a difference of 1 mm. in the arterio-venous CO_2 pressure corresponds to a difference of 0·4 volumes p.c. CO_2.

In order to consider the error in the minute volume caused by an error of a known magnitude in the determination of the arterio-venous

CO_2 pressure difference, take the CO_2 output to be a constant $= k/100$; then from equation (1),

$$mv = k \qquad \text{.....(4).}$$

Let n mm. = the error made in determining the arterio-venous CO_2 pressure difference.

From equation (3) this corresponds to an error of $\frac{2n}{5}$ volumes of CO_2 p.c.

The uncorrected minute volume (M) will be given by

$$M\left(v + \frac{2n}{5}\right) = k \qquad \text{.....(5).}$$

The percentage error (P) in the minute volume caused by such a change is given by

$$P = \frac{M - m}{m} . 100.$$

Substituting from equations (4) and (5) this becomes

$$P = \left[\frac{5k}{5v + 2n} - \frac{k}{v}\right] \cdot \frac{v}{k} . 100$$

$$= - \frac{200n}{5v + 2n}.$$

Therefore

$$P(5v + 2n) + 200n = 0 \qquad \text{.....(6).}$$

Or, expressing this in the usual coordinates,

$$x(5y + 2n) + 200n = 0 \qquad \text{.....(7).}$$

The curves for various positive and negative values of n corresponding to this equation are given in Fig. 1. Note that a positive error in the arterio-venous pressure difference causes a negative error in the minute volume, and *vice versa*.

In deriving these equations, k, the constant CO_2 output, has been eliminated. Hence, the above equations hold for any value of CO_2 output, and, consequently, for any magnitude of minute volume, so long as x expresses relative (percentage) errors.

For example, at an arterio-venous difference of 4 volumes p.c., an error of + 1 mm. causes a decrease in the minute volume of 9·1 p.c., and an error of − 1 mm. causes an increase of 11·1 p.c. over the correct value.

It is also possible to draw a curve to show the relation of the error (n) made in determining the arterio-venous CO_2 pressure difference to the percentage error (P) so caused in the minute volume, at any one value of the arterio-venous CO_2 difference (v).

A rearrangement of equation (6) gives

$$\frac{1}{P} + \frac{5v}{200n} + \frac{1}{100} = 0,$$

Or, expressing the variables in the usual coordinates,

$$\frac{1}{x} + \frac{5v}{200} \cdot \frac{1}{y} + \frac{1}{100} = 0 \qquad \text{.....(8).}$$

These curves for various values of v are given in Fig. 2.

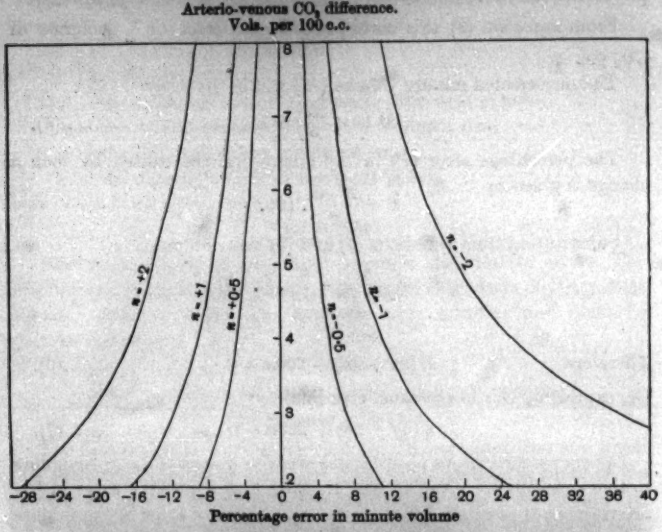

Fig. 1. The relation between the percentage error in the minute volume of the heart and the arterio-venous CO_2 volume difference, for different values of the error made in determining the arterio-venous CO_2 pressure difference (n in mm. Hg). Curves drawn to equation (7) in text.

Relative errors in the determination of the CO_2 output.

These are directly proportional. An error of 1 p.c. in measuring the CO_2 output will cause a 1 p.c. error in the value for the minute volume.

The foregoing shows that it is of paramount importance in the determination of the minute volume that great care should be taken to eliminate or reduce as far as possible all sources of error in measuring the difference in CO_2 partial pressures of the arterial and venous bloods; especially at low arterio-venous differences, such as exist at rest. However, the relative error rapidly decreases when the arterio-venous difference increases, as it does during exercise: this is fortunate as the

minute volume in exercise is greatly increased, making all errors tell, absolutely, to a greater extent.

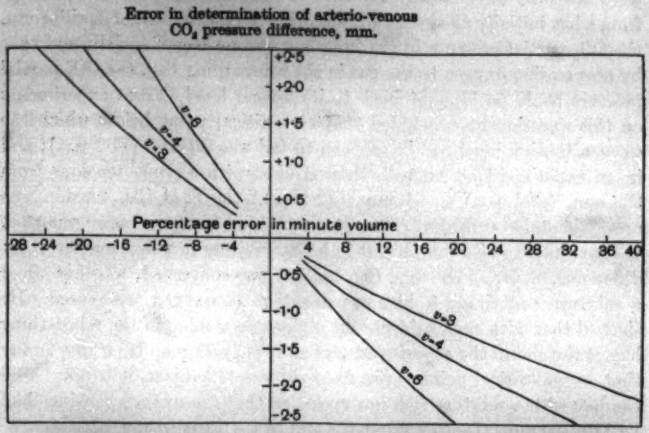

Fig. 2. The relation between the error in the determination of the arterio-venous CO_2 pressure difference and the percentage error in the minute volume of the heart, for different values of the arterio-venous CO_2 volume difference (v in volumes p.c.). Curves drawn to equation (8) in text.

The curves also show clearly that it is better to err on the side of making the arterio-venous CO_2 pressure difference too large rather than too small. This renders more important the proper oxygenation of the venous blood, as failure to carry this out properly leads to a too small arterio-venous CO_2 pressure difference.

In view of the foregoing the question at once arises as to what is the minimal pressure of oxygen permissible in the rebreathing bag to ensure full oxygenation of the blood during the rebreathing.

Henderson and Prince[9] and Meakins and Davies[12] used expired air in the rebreathing bags. Field, Bock, Gildea and Lathrop[5] and Brocklehurst, Henderson and Haggard[1] used mixtures of oxygen and CO_2; the former authors expressing themselves of the opinion that 150 mm. (19·7 p.c.) of oxygen, the final oxygen tension in their method, was sufficient fully to oxygenate the blood, and they give evidence to show that the blood taken from an artery during the last 5 sec. of a rebreathing period was 99 p.c. oxygenated.

Dautrebande(3) utilising the method of Meakins and Davies pointed out the error this method would lay itself open to if the blood were not fully oxygenated. He showed that after several rebreathings from a bag initially filled with air, following the attainment of equilibrium, the CO_2 partial pressure in the lung-bag system began to fall; and that by now adding oxygen to the gas in the rebreathing bag, the CO_2 partial pressure could be brought back to its former level. From experiments on this question he concluded that the critical point below which the oxygen tension must not be allowed to fall was 85 mm. (11·2 p.c.); and in an experiment on himself, showed that with oxygen tensions from 333 mm. (43·4 p.c.) to 94 mm. (12·4 p.c.) the final CO_2 tension was approximately constant. Results on three subjects are reported. Dautrebande also found that in some subjects it was a matter of indifference, as far as the final CO_2 tension was concerned, whether air or a mixture containing a higher percentage of oxygen were used. He showed that with such subjects the oxygen remaining in the rebreathing bag at the end of the experiment was more than 11 p.c. He states finally that as the critical point varies from subject to subject, it is best to fill the bag with a mixture rich in oxygen, so that the oxygen tension shall be 110–120 mm. (14–15 p.c.) at the end of the experiment.

The experiments undertaken by the writers on this point fall under two headings according to the method used. In the first place it was found that with methods such as that of Henderson and Prince, the oxygen in the rebreathing bag fell very rapidly and that it was consequently impossible to carry out a sufficient number of rebreathings to allow of proper CO_2 equilibrium being reached and, at the same time, to conserve a high final percentage of oxygen in the rebreathing mixture.

A modification of the Henderson-Prince method was therefore devised, in which the subject washed out his lungs with a mixture of suitable oxygen and CO_2 content before rebreathing. The apparatus is shown in Fig. 3. It enables the subject of an experiment to breathe into or out of any of three circuits at will without changing his position or removing his mouth from the mouthpiece. It consists of a brass slider (*S*) in which there are three circular apertures, provided with rims for the attachment of tubing. The slider fits into a holder which has one central aperture into which a glass tube is fitted, this acting as the mouthpiece (*M*). The slider is moved along until the aperture in it, to which is attached the required circuit, is opposite the mouthpiece. Correct alignment is facilitated by a spring catch (*C*), a projection on which fits into holes in the slider under each aperture. The screws (*P*)

act as stops, since, as they cannot be moved past *C*, they prevent the slider being moved too far.

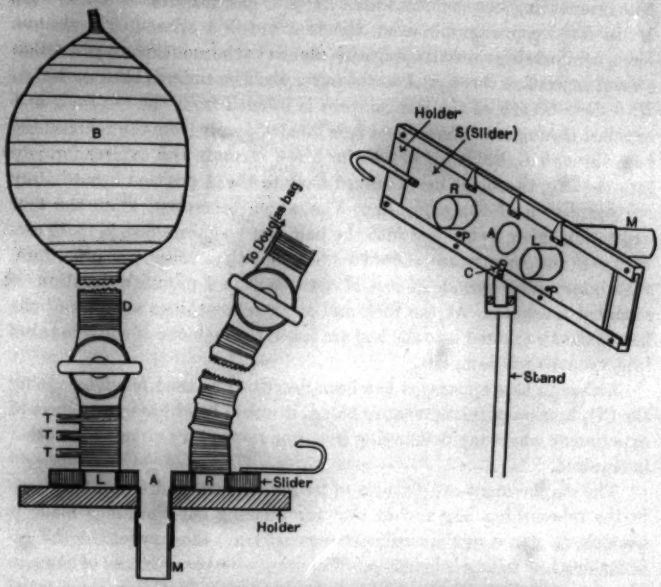

Fig. 3. Right: slider apparatus for changing breathing circuit.
Left: rebreathing bag and connections to slider.

The holder is made of sufficient length to close off the two apertures not in use. The opposing surfaces of the holder and slider are well greased with vaseline, to allow of easy movement and to render the apparatus airtight.

The small dead-space of the apparatus gives it a great advantage over a series of taps for respiratory work.

The apparatus is connected up as follows: the right-hand tube (*R*) is connected by means of a one-way tap to a Douglas bag which acts as a reservoir and is filled with a suitable gas mixture. The centre aperture (*A*) is left open. The left-hand tube (*L*) is connected to a rebreathing bag (*B*). This is a 4½ litre rubber bag provided with a wide rubber tube about 35 cm. long (*D*): between the bag and the slider is a one-way tap,

and also, as near to the slider as possible, side tubes (*T*) through which samples can be taken; these are closed with glass rods when not in use. The rebreathing bag is filled with 4 litres of gas mixture.

In making a determination, the taps are first opened. The subject being comfortably settled, applies his mouth to the mouthpiece and makes a deep expiration through *A* to the outer air. The slider is then moved to *R*; a deep breath of the gas mixture is inhaled from the reservoir and expelled through *A*. The subject now inhales deeply from the rebreathing bag, through *L*, holds his breath for a few seconds and expires forcibly into the bag, the slider being moved back to the *A* position immediately on completion of the expiration. The whole procedure, from the first expiration to air to the last into the bag, can be accomplished in 15 sec. with ease and, in any case, should not be longer than 20 sec. The procedure, with intervening periods of rest of not less than 1 minute's duration, is repeated six times. At the fifth and sixth rebreathings samples of the last of the air expired into the bag are taken through one of the side tubes into vacuous gas samplers.

Although this apparatus has been described as used for determining the CO_2 tension of mixed venous blood, it would be of use in any form of experiment where rapid changing from one respiratory circuit to another is required.

The employment of mixtures of 94 p.c. oxygen and 6 p.c. CO_2 both in the rebreathing bag and in that for washing out the lungs made it possible to carry out six rebreathings without the oxygen in the rebreathing bag falling below 60 p.c. By using suitable mixtures of oxygen, nitrogen and CO_2, the last-named being always in the proportion of 6 p.c., the final oxygen tension in the rebreathing bag could be approximately controlled. In practice, mixtures containing 94 p.c., 50 p.c. and 25 p.c. oxygen were used for the lung washing. The mixtures utilised in the rebreathing bag were either 6 p.c. CO_2 in 94 p.c. or 50 p.c. oxygen, or in air, a fresh sample of a particular mixture being used for each determination; or else starting with 94 p.c. oxygen, samples were taken after the fifth and sixth rebreathings, and then, after allowing the subject a short rest, the same mixture, now containing only about 60 p.c. oxygen, was used for a further period of six rebreathings in conjunction with a suitable washing mixture, in this case 50 p.c. oxygen: this procedure was repeated until the oxygen in the rebreathing bag became reduced to about 15 p.c. In this manner the effect of oxygen tensions at intervals from 60 p.c. of an atmosphere downwards could be tested.

Further experiments were carried out which omitted the intermediate

oxygen pressures and consisted only of estimating the CO_2 tension of the mixed venous blood with 94 p.c. oxygen and with air in the rebreathing bag; in the latter case the lung washing was dispensed with as it was unnecessary. The former determination gave a final oxygen tension of about 60 p.c. and the latter about 15 p.c. of an atmosphere.

The results of some of these experiments are given in Table I below:

TABLE I.

Date	Final oxygen tension p.c. atmosphere	Venous CO_2 tension mm.	Subject
4. iv. 1928	60	43·1	M. C. G. I.
	38	43·4	
23. iv. 1928	60	47·3	"
	35·4	47·5	
	26·2	47·0	
	14·5	46·0	
25. iv. 1928	60 (approx.)	45·6	"
	34	45·6	
	26	45·4	
	15·2	44·3	
26. vi. 1928	60 (approx.)	43·5	"
	15·1	42·2	
28. vi. 1928	60 (approx.)	45·0	"
	14·5	44·2	
27. iv. 1928	60	45·8	C. E. B.
	14·5	44·7	

From the results of these experiments it would seem that the oxygen tension in the air inspired from the rebreathing bag can be allowed to fall to about 26 p.c. without causing a lowering of the CO_2 tension of the mixed venous blood due to oxygen unsaturation of the blood; but that such an effect will be brought about if the oxygen tension falls as low as 15 p.c. of an atmosphere. The differences between the CO_2 pressures as determined with high or low oxygen pressures in the rebreathing bag, though small, were always in the same direction.

In the above experiments only two subjects were used. In order to examine more subjects and at the same time apply these findings to those methods which follow the recommendation of Laurens(10) in not holding the breath, but breathing to and from the bag during the rebreathing period, a second set of experiments were carried out.

The CO_2 tension of the mixed venous blood was determined by a method similar to that of Meakins and Davies(12). The apparatus consists simply of a rubber rebreathing bag as used before, with a two-way tap at the end of the 35 cm. tube and with a further short length of

rubber tube beyond this fitted with side tubes for sampling, and a glass mouthpiece.

The subject rests in a chair for at least 15 min. Then, with the nose clipped, he applies his mouth to the mouthpiece and expires as deeply as possible to air *via* the two-way tap. The tap is now turned to connect the mouthpiece with the rebreathing bag, which contains 4 litres of the required mixture. The subject breathes deeply three times in and out of the bag in about 12 sec., finishing with a deep expiration into the bag, when the tap is again turned to cut off the rebreathing bag. This procedure, with intermediate rest periods, is repeated six times in all. Samples of the last of the air expired into the bag at the end of the fifth and sixth rebreathings are taken into vacuous gas samplers through the side tubes provided. A sample of the gas in the bag can be taken through a convenient tube.

It was found that, starting with 94 p.c. oxygen in the rebreathing bag, the procedure reduced this finally to about 26 p.c. The experiments with each subject were therefore two determinations of the CO_2 tension of the mixed venous blood within a few minutes of each other; one starting with a mixture of 94 p.c. oxygen and 6 p.c. CO_2 in the rebreathing bag, and one with air and 6 p.c. CO_2 in the bag; the experiments being performed in varying sequence. Some representative results are given in Table II below:

TABLE II.

Date	Final oxygen tension p.c. atmosphere	Venous CO_2 tension mm.	Subject
13. vii. 1928	26·3	47·5	M. C. G. I.
	14·5	46·5	
17. vii. 1928	26·0	50·1	C. E. B.
	14·3	49·5	
12. ix. 1928	27·0	45·7	P. J. C.
	14·7	44·7	
14. ix. 1928	27·2	44·5	"
	14·3	42·5	
17. ix. 1928	26·4	47·0	F. S. W.
	12·3	45·0	
18. ix. 1928	26·1	48·3	"
	12·8	47·4	
18. ix. 1928	27·0	48·3	B. F.
	14·0	47·2	
20. ix. 1928	27·4	50·1	"
	15·7	49·3	

The results show that the venous CO_2 tension is higher in the subjects examined when the final oxygen tension in the rebreathing bag is 26 p.c.

than when it is allowed to fall to 14 p.c.; the difference varies from 0·6–2·0 mm., but the actual magnitude of the difference is of less importance than the fact that the change is always in the same direction. As a consequence it will be seen that, in order to avoid any effects of insufficient oxygenation of the venous blood in methods of this type, it is necessary to start the experiment with a gas mixture in the rebreathing bag consisting only of oxygen and CO_2.

Further evidence can be provided by an examination of the known data of the blood with regard to this effect of oxygenation. When air is inspired, it is diluted by the air remaining in the lungs and respiratory passages. Haldane(6) gives a table showing the relation between oxygen pressure in the inspired air and that in the alveolar air: some of these figures are reproduced in columns 1 and 2 of Table III. In column 3 are given the figures for percentage oxygen saturation of the blood at these alveolar oxygen tensions.

Now from the CO_2 dissociation curve the difference in tension exerted in the fully oxygenated and fully reduced blood by a given CO_2 content can be determined. It may be assumed that if these tensions $= o$ and r respectively, then the CO_2 tension at any intermediate value of oxygen saturation, say p p.c., will $= r + \frac{p}{100}(o - r)$. The CO_2 tensions thus calculated corresponding to representative values for venous blood, at the degrees of oxygenation given in column 3, are shown in columns 4 and 5.

The oxygen dissociation curve used is that given by Haldane(7): it is for blood in the presence of 40 mm. CO_2, consequently the effective oxygen pressures will be a little lower than the figures given in column 3, according to the actual CO_2 tension of the venous blood. The CO_2 dissociation curve used is taken from the same source(8).

TABLE III.

Oxygen pressure p.c. atmosphere			Pressure exerted by a CO_2 content of	
Inspired air	Alveolar air	p.c. oxygen saturation of the blood	55 vols. p.c. (mm.)	57·5 vols. p.c. (mm.)
80·24	72·21	100·0	48·0	54·2
63·67	57·57	100·0	48·0	54·2
20·93	14·50	96·5	47·6	53·7
15·65	10·60	94·7	47·4	53·5
12·85	8·34	89·0	46·7	52·7
11·09	7·10	84·5	46·2	52·1
6·23	4·30	55·0	42·7	48·1

The table shows that the differences are of a similar order to those determined experimentally and emphasises the necessity of using an

oxygen CO_2 mixture for rebreathing experiments of the type under consideration.

It should also be noted that Meakins and Davies(11) found that with normal subjects breathing ordinary air (20·93 p.c. oxygen) the arterial blood was 95 p.c. saturated with oxygen, and that breathing a mixture containing only 12·6 p.c. oxygen the saturation was reduced to 81·4 p.c. The deeper breathing in rebreathing experiments probably allows of better equilibrium being reached and therefore slightly higher saturation with oxygen ensues.

No attempt was made to assign experimentally any value to the "critical point," *i.e.* that tension below which the oxygen in the rebreathing bag must not be allowed to fall. Owing to the shape of the oxygen dissociation curve the reduction of CO_2 tension (assuming the CO_2 content to remain constant) with falling oxygen tensions starts very gradually, so gradually as to be undetectable by any methods which are applicable to the human subject. The experimental data show that this "critical point" may be lower than 26 p.c. of an atmosphere (198 mm.) but is certainly higher than 14 p.c. (106 mm.). This, and the data in Table III, incline the writers to take 25 p.c. atmosphere (190 mm.) as a representative value for the critical point; so long as the final oxygen tension in the lung-bag system is not less than 25 p.c. of an atmosphere, the blood may be assumed, for all practical purposes, to be fully oxygenated.

The results obtained differ considerably from those of Dautrebande already quoted in indicating a much higher critical oxygen tension, 190 mm. as against 85 mm., or 120 mm. It is difficult to see why Dautrebande obtained such low results; reference to Table III shows that even at his safety point, 120 mm. (15·8 p.c.), there is sufficient oxygen unsaturation to cause a definite fall in the CO_2 tension of the mixed venous blood, and in this table it has been assumed that complete equilibrium between the lung and blood gases is attained. The results of Meakins and Davies(11) given above point to a similar conclusion.

Summary.

1. Attention is drawn to the effects of insufficient oxygenation of the lung blood in rebreathing experiments for determining the CO_2 content of the mixed venous blood, and to their import in the application of the values obtained to the calculation of the minute volume of the heart's output.

2. An analysis is made of the absolute and relative errors in the derived values.

3. A method for carrying out rebreathing experiments without a very rapid lowering of the oxygen tension in the rebreathing bag, and a method for rapid changing from one respiratory circuit to another are described.

4. As a result of these, and a second series of experiments, it has been found that to ensure full oxygenation of the mixed venous blood the oxygen tension in the lung-bag system should not be allowed to fall below 25 p.c. of an atmosphere.

5. The necessity of beginning any series of rebreathing with a mixture of oxygen and CO_2 is demonstrated. The use of air and CO_2, or still more so, expired air, leads to errors in the values calculated for the minute volume of the heart which range up to 50 p.c. in the resting state.

6. Data from the known properties of blood are given to support this view.

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THE EFFECT OF MUSCULAR EXERCISE ON THE OXYGEN CAPACITY OF THE BLOOD OF MAN.

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In the course of some studies of muscular activity at low oxygen pressures we have had occasion to study the oxygen content of the blood. Before and during exercise blood was drawn from the dorsum of the hand which was immersed in water warmed to 45–47° C. Goldschmidt and Light(1) and Mrs Kerridge(2) have shown that blood obtained in this way approaches arterial blood in composition. The exercise consisted in alternately sitting and rising to the erect position at a rate of approximately 30 times per min. In some of the experiments the subject breathed air; in others the inspired gas was an air-nitrogen mixture containing 10–15 p.c. oxygen. The oxygen content and capacity of the blood were determined by the method of Van Slyke and Neill(3).

Table I. Oxygen content and capacity of blood during rest and exercise.

			Rest			Exercise		
Date	Subject	p.c. O_2 in gas	O_2 cap.	O_2 cont.	p.c. sat.	O_2 cap.	O_2 cont.	p.c. sat.
March 26	S.	12·00	16·85	12·20	72·4	19·21	16·72	87·0
April 4		12·25	16·53	16·06	97·0	17·81	15·25	86·0
„ 6		11·36	17·28	14·95	86·5	17·82	12·91	72·4
„ 10		20·93	16·52	16·08	97·4	17·63	17·32	98·3
„ 10		20·93	(16·73)	(16·20)	(96·9)	—	—	—
May 4		12·50	—	14·11	73·4	20·09	13·75	68·6
„ 4		12·50	(19·26)	(15·52)	(80·6)	—	—	—
„ 8		20·93	18·39	17·37	93·6	18·79	18·05	96·2
March 28	R.	—	22·48	18·18	80·8	23·66	20·56	86·7
April 5		11·36	22·39	18·85	84·2	23·34	17·81	76·6
„ 23		13·00	20·62	17·12	82·9	21·30	15·30	71·2
„ 23		13·00	—	(18·05)	(87·5)	—	—	—
May 7		20·93	21·39	20·75	97·1	21·31	21·12	99·1
„ 9		10·50	21·75	16·44	75·6	22·08	16·20	73·4
„ 9		10·50	(21·68)	(16·51)	(76·2)	—	—	—
„ 17		20·93	21·42	20·00	93·3	21·78	21·02	96·5
March 27	I.	11·80	20·54	15·38	75·0	20·28	16·50	80·9
March 28	L.	14·92	20·05	18·95	94·6	20·92	19·95	95·4

Note. Figures in parenthesis are for blood obtained by arterial puncture. Samples for other figures were taken from the hand immersed in hot water.

The results of the experiments are shown in Table I. The most constant finding was an increase during exercise of the oxygen-carrying power of the blood. This occurred in 12 of 14 experiments, amounting usually to somewhat less than 1·00, but in one case rising to 2·36 volumes p.c. In the subject S. the increase was approximately the same whether air or an oxygen-poor mixture was breathed. With R., on the other hand, the rise in oxygen capacity induced by exercise appears to be greater when anoxemia is present.

The data on the oxygen saturation of the blood are less uniform than those dealing with oxygen capacity. Our findings when the subject breathed air confirm those of other investigators, that the blood obtained from the veins of the hand immersed in hot water has approximately the same oxygen content as has the true arterial blood. However, when the subject breathes a gas mixture containing 10–15 p.c. oxygen, the blood from the hand veins may have a distinctly lower oxygen content than the true arterial blood. This phenomenon is of some interest as it suggests that the observation made on dogs by Bronk and Gesell(4), namely that there is increased carotid and decreased femoral flow during anoxemia, may also apply to man. Schneider and Truesdell(5) measured the blood flow through the hand during anoxemia and found a decrease. Barcroft, Bock and Roughton(6) found that the circulation through the hand was greatly decreased in a case of paroxysmal tachycardia. On the other hand, Harrison and his co-workers(7) found the total blood—minute cardiac output—of normal, unanæsthetised dogs to be increased when the arterial blood is less than 75 p.c. saturated. Our findings, as well as those mentioned above, suggest that the circulatory compensation to anoxemia consists in changes in distribution as well as in the amount of blood flow.

Because of the close agreement between our values for arterial and hand blood while the subject breathed air, we are inclined to place confidence in our results, which indicate that during exercise while breathing air there is a tendency for the saturation to be somewhat greater than at rest. Eppinger, Kish and Schwarz(8) have reported similar observations. Harrop(9) has also studied this subject. But when the subject breathed oxygen-poor mixtures the findings concerning percentage saturation were variable, and since the hand blood may be as much as 5 p.c. less saturated than the true arterial blood, we are not inclined to attach great significance to our figures for arterial saturation during anoxemia. The data as they stand indicate that the arterial saturation may increase but usually decreases during exercise at low oxygen

pressures. In order to be certain of this point it is necessary to obtain blood from the artery during exercise, and this we have not been able to do.

At present we are unable to make any definite statement as to the cause of the increase in the oxygen capacity. In addition to the many workers who have studied the osmotic pressure of excised muscle it was shown by Barcroft and Kato(10) on mammalian muscle, and by Back, Cogan and Towers(11) on frog's muscle, that a muscle provided with an efficient circulation gained in weight on continued stimulation, imbibing water presumably from the circulation through its vessels. On the other hand, recent work on the spleen suggests that the rise in oxygen capacity during exercise is in part, at all events, due to the contraction of the spleen inasmuch as a similar rise in animals is reduced or abolished by splenectomy. In the horse and dog, where the rise in oxygen capacity is much greater than in man, the experiments of Scheunert and his associates(12) yielded evidence of combined splenic and osmotic effect.

Since the completion of these experiments and while this report was in preparation a paper by Bock, Vancaulaert, Dill, Fölling and Hurxthal(13) has appeared. These investigators also found an increase in the oxygen capacity of the blood of human subjects during exercise.

Summary.

1. Blood from the hand veins after immersion in water at 45° is indistinguishable in oxygen content from arterial blood while the subject is breathing air.

2. It is not indistinguishable but is of slightly lower oxygen content in anoxemia.

3. The oxygen capacity is increased during exercise while the subject breathes either air or an oxygen-poor mixture.

4. The oxygen saturation of the arterial blood sometimes goes up and sometimes down during exercise in air, but more often it goes up.

In conclusion we wish to express our thanks to Prof. Barcroft for his interest and advice.

The expenses have been born largely by a grant from the Medical Research Council.

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THE HYDROGEN ION CONCENTRATION OF THE ISOLATED UTERUS.

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Introduction. The original aims of this research were (1) to determine whether the hydrogen ion concentration of minced tissue could throw light on the responses of plain muscle to changes in the hydrogen ion concentration of Ringer solutions in which it is immersed; and (2) to find whether any difference in the *c*H of the tissue, associated with relaxation and contraction, could be detected. Although the first aim has not been realised, the pursuit of it led us to experiments on the *c*H of isolated plain muscle, minced after immersion in Ringer solutions of various compositions. The results of these experiments were unexpected, and, in our opinion, of sufficient general interest to place on record. The second aim was carried out, and the results obtained are described.

Technique. The *c*H measurements were by the glass electrode method (1), the treatment of the tissue being essentially the same as described in a paper by one of us with Furusawa (2) on the hydrogen ion concentration of the muscles of the cat. The uterus of the cat was used for the greater part of this investigation, because its size is as small as can easily be adapted to the glass electrode method without undue error, and as large as may allow reasonably rapid diffusion between the interior of the tissue and the solution bathing its surface. In some experiments (as will be indicated) it was slit longitudinally to aid such diffusion.

The *c*H results found for such a minced tissue correspond to the hydrogen ion concentration of the liquid phase of the moist mince, which must consist of the fluid from the ruptured cells together with the tissue juices. Loss of carbon dioxide from these liquids cannot be prevented during the mincing process, and any difference in the rate of loss between one sample and another must be reckoned as an experimental error. It is realised that no deductions as to the absolute hydrogen ion concentration inside the living cells can be made from

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such measurements on minced tissues, but it seems probable that differences in the observed values are significant, and no method is at present known to us whereby closer indications of *c*H differences within the cell membrane can be obtained. A further point is that the tissue includes, in addition to plain muscle, variable amounts of connective tissue and a considerable relative amount of mucous membrane. We are unable to say to what extent the results we have found are due to variations in the *c*H of these structural components of the uterus, but since the muscular tissue is greatest in amount, it is probable that the *c*H of the minced tissue is determined principally by it.

The *p*H measurements of the solutions were made under liquid paraffin and at room temperature, which varied on different occasions between 15° and 18° C.

The cats were anæsthetised with ether. In the earlier experiments they were bled by cutting the carotid artery. The uterus was gently dissected, and the horns attached to threads or glass hooks at the ovaries and through the muscle near the junction. The two horns were used separately, one frequently as a control. In the earlier experiments each horn was slit longitudinally before immersion in the saline bath. About 50 c.c. of the saline solutions were contained in boiling tubes, and warmed by a water bath at 37° C. Air was led into the solution through a glass tube which ended in a hook, to which the lower end of the uterus could be attached. The upper end was attached to an isotonic lever. Variations in detail of the foregoing technique took place as experiment necessitated, and are noted below.

The solutions were made up freshly each day from British Drug Houses A.R. chemicals. The standard concentrations, in all solutions, of sodium, potassium, and calcium chloride respectively, were 0·9 p.c., 0·042 p.c., and 0·024 p.c. as in Burn and Dale's(3) solution. Sodium bicarbonate or phosphate was added in varying amounts as mentioned below.

Experimental results.

The hydrogen ion concentration of the saline solutions. As a preliminary to the measurements of the *c*H of the uterus under various conditions, some experiments were made on the hydrogen ion concentration of the salt solution of the following composition:

Table I. Salt solution.

NaCl	0·9 grm.
KCl	0·042 ,,
$CaCl_2$	0·024 ,,
$NaHCO_3$	0·05 ,,
H_2O	100·0 c.c.

This corresponds to the solution used by Burn and Dale(3) with the omission of magnesium chloride and dextrose. The results are given in the following table:

TABLE II. *p*H of salt solution.

Conditions	*p*H	
	Uterus absent	Uterus present
Freshly made up	7·55 ±0·03 (3)	—
After ¼ hour air bubbling at 37° C.	7·78 ±0·04 (7)	7·84 ±0·07 (8)
After ½ hour–2 hours' air bubbling at 37° C.	8·14 ±0·06 (8)	8·01 ±0·09 (10)
After standing 6 hours at room temperature	8·05	—

The figures given represent mean values and the mean deviations, and the numbers in brackets indicate the number of observations.

From the above figures it will be seen that such a solution becomes more alkaline either on standing in air, or on bubbling air through it, as would be expected owing to loss of carbon dioxide. No significant difference could be observed between the effect of half an hour's and two hours' bubbling, but a difference could be seen between that of a quarter of an hour and half an hour. The final *p*H attained seemed to be independent of the presence of a uterus in the solution, within the limits of experimental variation.

Calcium in saline solutions. The relatively small solubility of calcium carbonate and phosphate is of great importance in the consideration of physiological saline solutions in which the hydrogen ion concentration is varied. Calcium carbonate is much less soluble than calcium bicarbonate, and tri-calcium diphosphate much less soluble than calcium monohydrogen phosphate; consequently the solubility products of calcium carbonate and tri-calcium diphosphate respectively are the limiting factors, although the absolute amounts of CO_3'' and PO_4''' ions present in solutions at a *p*H less than 8·0 are small compared with the concentrations of HCO_3' ions and HPO_4'' ions.

It can be inferred from the constants given by Holt, Le Mer and Chown(4) and Hastings, Murray and Sendroy(5) that in the solutions used by us—*i.e.* (i) a modified Burn and Dale solution, (ii) a similar solution containing *M*/250 bicarbonate, and (iii) a solution containing *M*/250 phosphate instead of bicarbonate, as used by Evans and Underhill(6)—the products $[Ca^{\cdot}]\,[CO_3'']$ and $[Ca^{\cdot}]^3\,[PO_4'']^2$ respectively exceed the solubility products of these salts above *p*H 8·1, 7·5 and 5·7 respectively. That is, the solutions are supersaturated with respect to calcium carbonate or phosphate on the alkaline side of these figures. Solution (ii) for example, at *p*H 8·2, would have a calcium ion concen-

tration five times the equilibrium value. It was pointed out by the above workers and also by Van Dyke and Hastings(7) that similar saline solutions could remain supersaturated with respect to these substances for days. The reason for this condition of delayed equilibrium is not apparent, and it is not easily possible to determine chemically whether the excess calcium is ionised, or is combined as carbonate or phosphate, but not precipitated. Van Dyke and Hastings claim to have some indication of the state of the calcium by observing the response of a guinea-pig's uterus to constant doses of pituitary extract when placed in solutions in which all the ionic concentrations were maintained constant, with the exception of that of calcium. In this way they used the uterus as a "biological calcium electrode" and believe that the calcium in the supersaturated solutions is in an ionic form. Therefore, in view of this work, and on account of the lack of direct evidence, it may be assumed for the present that in the saline solutions under consideration the calcium is in the ionic condition, and that in spite of the absence of true ionic equilibrium its ionic concentration is constant during the course of an experiment with freshly prepared solutions, lasting only a few hours. In the case of the phosphate solutions this is only true at values of *p*H less than 7·8, at which point precipitation takes place.

Assuming that the bicarbonate solution of the composition given in Table I is in equilibrium with the CO_2 in atmospheric air (0·04 p.c.), after half an hour's air bubbling, when the *p*H has been found to be 8·1, the amount of total carbonate in the solution can be calculated to be 0·001 *M*. The amount of $NaHCO_3$ added corresponded to 0·006 *M*. Hence it follows that 83 p.c. of the carbonate has been removed during aeration, and the buffering power of the solution must be very small. This solution however is not supersaturated with regard to calcium under these conditions.

The differences of hydrogen ion concentration between the isolated uterus and its solution. If a uterus be removed from an anæsthetised cat, and placed in oxygenated saline solution of the composition described above, there is a striking and permanent difference between the hydrogen ion concentration of the muscle and that of the liquid bathing it. The muscle is invariably more acid than a relatively alkaline solution, as is shown in Table III, the difference of *p*H amounting to about 1·1. Measurements given in the table were made on the muscles, and on the solutions in contact with them at the time of their removal. The durations of immersion in the saline solution at 37° C. with air bubbling are

recorded, and the *p*H values of the muscle show no significant variation with time.

TABLE III. *p*H of cat's uterus in aerated bicarbonate buffered saline solutions at 37° C.

*p*H of uterus	*p*H of solution	Δ*p*H	Duration of immersion
7·21	(8·01)	+0·78	2–3 sec.
6·73	(8·01)	+1·28	2–3 sec.
6·89	7·72	+0·83	15 min.
6·90	7·84	+0·94	30 min.
6·94	8·13	+1·19	1 hour
6·60	7·72	+1·12	1 hour 40 min.
6·75	8·08	+1·33	2 hours
6·83	7·95	+1·12	2 hours 10 min.
6·80	7·95	+1·15	2 hours 20 min.
6·83	7·94	+1·11	2 hours 50 min.

A comparison of the values of *p*H of the muscles in contact with the above bicarbonate buffered solution (Table III) with those of muscles in contact with more acid solutions of similar composition (Table VI), indicates that the hydrogen ion concentration of the uterus is largely independent of that of the solution in which it is suspended. Muscles immersed in the acid media are indeed more acid than those in alkaline media, but the change in *p*H of the muscle is only about one-quarter of that in the corresponding solutions, and is barely outside the range of variation of individual uteri.

Similar observations on the conditions of uteri in salt solutions buffered with phosphate are recorded in the following table:

TABLE IV. *p*H of cat's uterus suspended in aerated phosphate buffered saline solutions at 37° C.

*p*H of uterus	*p*H of solution	Δ*p*H	Length of uterus	Duration of immersion	Concentration of buffer
6·99	7·32	+0·33	Contracted	15 min.	0·004 *M*
6·88	6·74	−0·14	Relaxed	15 „	„
6·68	6·12	−0·56	„	15 „	„
6·79	6·12	−0·67	„	15 „	„
6·67	6·62	−0·05	Contracted	30 „	0·001 *M*
6·63	6·07	−0·56	Relaxed	30 „	„
6·59	6·90	+0·31	Intermediate	1 hour	„
6·34	6·90	+0·56	„	1 „	„
6·56	6·90	+0·34	„	2½ hours	„
6·59	6·90	+0·31	„	2½ „	„
6·62	7·05	+0·43	Relaxed	4 „	„
6·82	7·03	+0·21	„	6 „	„

Here again duration of immersion in the solution appears to be without marked influence on the *p*H of the muscles. The muscle seems even more independent of the hydrogen ion concentration of the phosphate buffered solution than it is of that of the bicarbonate buffered solution.

The *p*H of the horn of a uterus taken straight from the cat, after ether anæsthesia and bleeding, was found to be 7·07 ± 0·07. The above *p*H values of the muscles after immersion are lower than this figure, indicating that acid is developed in the course of the manipulation. The stage at which this acid is formed will be discussed in a later section.

The hypothesis that the excitability of heart muscle is influenced by the difference of *p*H between the muscle and its environment (Andrus(8)) has a suggested application to unstriated muscle (Lovatt Evans(9)). We therefore examined our figures, in the hope of detecting some correlation between the degree of contraction of the uterus and the difference of hydrogen ion concentration between the muscle and the solution. If attention be confined to the cat's uterus, which tends to contract in alkaline and relax in acid media within the physiological range of *p*H, there does perhaps appear to be some such qualitative relation. The greatest differences of hydrogen ion concentration were found after immersion in bicarbonate solutions of *p*H about 8·0, in which the muscles were contracted. However, we are not inclined to regard this relation as significant but as a consequence of our happening to choose solutions which varied more widely in an alkaline sense, than in an acid sense, from the mean *p*H of the uteri. Several examples are recorded in Tables IV and VI in which a muscle is appreciably more alkaline than the solution, and although the difference of *c*H was considerable, there was no indication of contraction. Moreover, consideration of the behaviour of the guinea-pig's uterus throws further doubt on Andrus's hypothesis. This organ is commonly relaxed when in equilibrium with bicarbonate buffered solutions at a *p*H of about 8·0.

Table V. *p*H of the guinea-pig's uterus and of bicarbonate buffered solutions, after ½ hour immersion at 37° C.

*p*H uterus	*p*H solution	Δ*p*H
6·96	7·91	+0·95
6·76	8·27	+1·51
6·94	8·31	+1·37

Table V shows that the differences of *p*H between the modified Burn and Dale solution and the guinea-pig's uterus are of the same order as found with the cat's uterus. The uteri were taken from guinea-pigs 350 grm. in weight, and were not quite fully relaxed in the solution. Uteri from younger animals, which relax more perfectly in such circumstances, are so small that they warm up quickly in the glass electrode during measurement of their hydrogen ion concentration, and the

consequent acid formation introduces large errors. Experiments performed on them are therefore not recorded.

Comparison of conditions in bicarbonate and phosphate buffered solutions of the same pH. In a further series of experiments on cat's uteri, one horn of a uterus was placed in a bicarbonate buffered solution, the other horn in a phosphate buffered solution of initially the same pH. Concentrations of sodium, potassium and calcium chlorides were the same in all solutions. The hydrogen ion concentrations of the bicarbonate buffered solutions were adjusted by bubbling suitable mixtures of carbon dioxide and air through them throughout the experiment, and diffusion at the surface of the liquid was delayed by a one-inch layer of liquid paraffin. Air was bubbled through the phosphate buffered solutions at about the same rate. Concentrations of buffer at the pH chosen were calculated to give 0·004 *M* total carbonate and total phosphate respectively. The results are given in Table VI.

TABLE VI. pH of two horns of cat's uteri immersed in bicarbonate and phosphate buffered solutions.

Cat No.	pH of uterus	pH of bicarbonate solution	ΔpH	pH of uterus	pH of phosphate solution	ΔpH	Length of uteri	Size of uterus
1	6·66	7·44	+0·72	6·80	6·94	+0·14	Intermediate	Large
2	6·77	7·34	+0·57	6·56	7·19	+0·63	Contracted	Large
3	6·48	7·02	+0·54	6·57	6·97	+0·40	Relaxed	Medium
4	6·37	6·95	+0·58	6·40	6·95	+0·55	Intermediate	Small
5	6·67	6·41	−0·26	6·35	6·35	±0·00	Relaxed	Large
6	6·47	6·29	−0·18	6·86	6·32	−0·54	Relaxed	Large

In experiments on cats Nos. 1, 3, 5 the horn of the uterus first removed was placed in bicarbonate solution; in cats Nos. 2, 4, 6 the first horn was placed in the phosphate solution. The periods of immersion were one half hour except in cat No. 1, where the period was one and a half hours for reasons external to this experiment.

These figures lend no support to the view (Jacobs[10], Scott[11], Dale and Evans[12], Hartree and Hill[13], and others) that the cell membrane is more permeable to carbonic than to phosphoric acid, and fit in better with the finding of Stella[14], that inorganic phosphate is able to pass freely by diffusion in and out of a muscle immersed in Ringer's solution.

Development of the acid in the uterus during isolation. It has been shown above that acid is formed during the manipulation of the uterus, and that the consequent pH change is irreversible. Variations in the technique were introduced in an attempt to discover at what stage

this acid formation took place. Substitution of chloralose for ether as anæsthetic had no significant effect on the results. In experiments in which the cat was not bled previous to the removal of the uterus the acid production was frequently less, but not absent. The practice of slitting each horn of the uterus before introduction into the saline bath had no detectable effect on the final *p*H of the uterus. That some of the acid formation could take place in the animal was shown by dissecting the uterus, ligaturing one horn, removing it and plunging it in liquid air, and repeating the procedure with the other horn of the uterus after an interval of five minutes. The second horn was often more acid than the first by varying amounts up to 0·60 *p*H. That the majority of the acid formation took place previous to immersion in the saline bath was shown in experiments in which one horn of the uterus was put straight from the cat into liquid air, while the other horn was tied on to the glass hook, dipped in the saline solution at 37° C., taken out again immediately, cut off the hook, and transferred to liquid air. In one experiment the *p*H of the first horn was 7·15 and the second horn 6·73, and the time between administering the anæsthetic and placing the uterus in liquid air was 5 min. 10 sec. for the first horn, and 7 min. 58 sec. for the second horn. The time interval between the ligaturing and the liquid air immersion was 30 sec. for the first horn and 1 min. 40 sec. for the second horn. In this experiment the uterus was smaller than the average. Other experiments indicated that the acid formation was less rapid in larger organs. The experimental results were also found to be unaffected when oxygen was substituted for air bubbling.

It was therefore concluded that the acid formation took place between the time of ligaturing the uterus and the immersion in the saline bath, that it was not affected by the injury due to slitting, nor by the use of ether as an anæsthetic, and that it tended to be greater when the animal had been bled previously.

Post-mortem acid formation. Measurements were made on uteri in which post-mortem acid formation was allowed to develop to a maximum. In some cases the organ was left at room temperature overnight between two watch glasses (to prevent excessive drying), and in others it was placed in a covered boiling tube in a water bath at 37° C. for three to four hours. The results varied between 6·22 and 7·03, the mean of 16 determinations being 6·58. This value is more alkaline than that found by Furusawa and Kerridge(2) for the cardiac and gastrocnemius muscles of the cat under similar conditions. The high degree of variation may be due to the variation in buffering power of the uterus as shown

by the same workers, and is not greater than would be accounted for on that ground only. The above *p*H value would correspond to a lactic acid increase of about 1·2 millimols (or 0·11 grm. p.c.) above the resting value. This figure is of the same order as that found by Lovatt Evans[15] for the lactic acid content of the cat's uterus by analytical estimation of the lactic acid.

The influence of state of contraction or relaxation on the hydrogen ion concentration of the uterus. The possibility of acid formation associated with contraction was first investigated on cats anæsthetised with chloralose. One horn of the uterus was removed before, and the other after, injecting post-pituitary extract into the jugular vein; the results are shown in Table VII.

TABLE VII. *p*H of the cat's uterus before and after injection of post-pituitary extract.

*p*H before injection	*p*H after injection	Interval between injection and removal of second horn	Dose (international units)
7·00	6·99	5 min.	0·06
7·31	7·04	5 „	0·06
6·60	6·61	10 „	0·12
7·46	7·46	10 „	0·12
7·24	7·41	10 „	0·12
7·46	7·58	10 „	0·12
7·37	7·50	10 „	0·12

Thus no acid formation was detected in response to stimulation of the uterus of the anæsthetised cat with doses of pituitary extract sufficient to ensure full contraction for at least an hour (Knaus[16]). A similar experiment on a rabbit gave 6·72 and 6·80 as the values of *p*H for a half uterus before and after pituitary injection. If acid be formed, it may be rapidly removed by the circulation through the muscle of so adequately buffered a liquid as blood. It was determined therefore to attempt analogous experiments on the uterus suspended in lightly buffered saline solutions. Cats' uteri were suspended in about 50 c.c. of the standard saline solution buffered with 0·001 *M* phosphate, adjusted to *p*H 7·22. After some hours' immersion 0·5 c.c. of a suitable concentration of a post-pituitary extract (neutralised to *p*H 6·82) was added to one horn; after 10 minutes' contraction both the contracted and unaffected horns were removed to liquid air for *p*H determinations. The results are given in Table VIII.

Similar experiments were performed on the uteri of two young (1½ kilo) rabbits which were stimulated for 5 min. with epinine[1], and

[1] See note to Table VIII.

TABLE VIII. Comparison of the *p*H of contracted and relaxed isolated uteri.

	*p*H of relaxed uterus	*p*H of solution	*p*H of contracted uterus	*p*H of solution	Stimulus
Cat 1	6·51	7·06	6·82	7·03	Pituitary
Cat 2	6·69	7·04	6·62	7·05	Pituitary
Rabbit 1	6·68	7·16	6·82	7·32	Epinine* ($1:10^5$)
Rabbit 2	6·82	7·42	6·82	7·30	Epinine

* Epinine (3 : 4—dihydroxy phenyl-ethylmethylamine) is a stable substitute for adrenaline produced by Burroughs, Wellcome, Ltd. The activity of epinine is about one-tenth of that of adrenaline. The dose chosen gave about maximal contractions of the uterus.

these results are also recorded in Table VIII. In this case the solution was oxygenated and buffered with 0·004 *M* phosphate. After the relaxed muscles were removed to liquid air a sample of the solution was taken, and its *p*H is given in the table. To the rest of the solution the standard dose of epinine was added, the liquid allowed to remain with continued oxygenation for 5 min., and samples then taken for *p*H determinations gave the values 7·23 and 7·30 respectively.

It is evident that no conclusions can be drawn from the results of this method. Acid formation, if any, due to contraction, has not revealed itself either by marked acidity of the muscle or by marked acidity of the liquid in the bath which might occur if the acid were to diffuse rapidly out of the muscle. Inspection of Tables III and V, in which the states of contraction and *p*H values for uteri are given, reinforces the conclusion that no striking variation of *p*H of muscle is associated with contraction or relaxation of the muscles. The variation, if any, is masked by adventitious *p*H differences between different samples of muscle.

SUMMARY.

1. The *p*H of the minced uterus is very little affected by that of the solution in which it has been immersed. In the usual bicarbonate-buffered, aerated, saline solutions at a *p*H of about 8·0 the difference in *p*H between muscle and solution is about 1·1.

2. The effect of the *p*H of a solution on the tone of a uterus cannot be attributed to the sign or magnitude of the difference of *p*H between the muscle and the solution.

3. The variation of calcium ion concentration with *p*H of certain bicarbonate and phosphate buffered Ringer solutions is discussed.

4. Measurements on the minced tissue indicate that the hydrogen ion concentration of an isolated uterus is greater than that of the uterus *in situ*.

5. The acid formation, represented by this difference, is largely irreversible. It takes place during excision and before immersion in saline solutions.

6. After maximum post-mortem acid formation, values of pH of 16 samples of cats' uteri (minced) varied between 6·22 and 7·03—the mean value being 6·58.

7. Comparison of uteri before and after stimulation with drugs, both *in vivo* and *in vitro*, yielded no evidence of acid formation associated with contraction.

In conclusion we beg to offer our thanks to Prof. C. Lovatt Evans for advice and to the Medical Research Council for defraying the expenses of this research.

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STUDIES IN THE PULMONARY CIRCULATION.

II. The action of adrenaline and nicotine.

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Adrenaline, in so far as its vascular effects are concerned, produces the same type of action as stimulation of vasomotor nerves. On the pulmonary vessels its cardiac action masks any trifling direct effect it may have, as Wiggers(1) pointed out; and we have shown that its action is seen equally well after the cardiac nerves are divided and after the autonomic ganglia are paralysed by apocodeine.

Schafer and Lim(2) thought that because intravenous injections of adrenaline caused a rise in pulmonary arterial pressure before a rise in systematic pressure whilst the reverse sequence obtained when the injections were made through the carotid artery into the aorta, was evidence of constriction of the pulmonary vessels; but this reasoning we do not consider valid on grounds similar to those of Wiggers(1). It is quite possible, as we have demonstrated elsewhere, to double the pulmonary pressure without altering the systematic pressure, or the tone of the systemic or pulmonary vessels to any appreciable degree. This effect is due to dilatation of the coronary vessels. Anrep and Stacey and others(3, 4) have made it clear that the action of adrenaline on the coronary vessels in dogs and rabbits is dilatation, and this together with the direct effect on the right heart accounts for the primary rise in pulmonary pressure. In other words the early rise in pulmonary pressure in the intact animal is of the same nature as the rise in pulmonary pressure in Führer and Starling's heart-lung preparations(5). Neither of them affords evidence of pulmonary vaso-constriction. This fact should be borne in mind in the interpretation of changes in the vascular lung-volume under adrenaline.

Nicotine, since the work of Langley and Dickinson(6), has been used by physiologists to affect autonomic ganglion cells, and it is often assumed that stimulation and paralysis of these and the cerebrospinal axis are its only significant actions. The action is on motor cells and not

sensory; this has been shown for both the spinal cord and posterior roots of spinal ganglia (7). The action has been further localised on the "cell substance" and not on the preganglionic arborisation (7).

Nicotine has another action which is not so well recognised. It excites plain muscle when ganglion cells are believed to be absent. Thus its action on the isolated perfused heart is not essentially different from that on the intact heart in the living animal with the vagi cut (8), although sympathetic ganglion cells are believed to be few or absent. It constricts coronary vessels (9) when adrenaline either dilates or is without action, and it constricts isolated and perfused vessels of the frog (10) and of the rabbit's limb and intestine. Nicotine constricts the bronchioles in surviving lungs (11); it constricts pulmonary vessels under conditions when adrenaline dilates them (12); and it constricts isolated vein rings (13) where ganglion cells are not found (14). The retractor penis of the dog, which according to Fisher contains no ganglion cells, is also contracted by nicotine (15). Clearly then, a good deal of evidence exists to show that nicotine acts not only on ganglion cells, but on more peripheral structures also. Since its effects on the heart and systemic blood-pressure are practically identical with those of adrenaline, it affords a useful substance with which to compare the action of adrenaline on the pulmonary circulation.

Experimental.

In all these experiments cats or dogs were anæsthetised with A.C.E. and urethane or chloralose and the vagi cut. Doses of nicotine and adrenaline, which produced similar effects on the blood-pressure and heart, were injected intravenously at a standard rate. The relative amounts required for such comparison are for cats in the ratio of one part of adrenaline to 2·5 parts of nicotine, approximately. The effects are so closely similar, when the doses are carefully graded, that it is impossible to say from examination of blood-pressure and cardiometer records which of the two alkaloids has been administered. Nevertheless, perfusion experiments through the pulmonary and coronary vessels make it abundantly clear that these two substances act differently. In the case of pulmonary vessels whilst the constrictor action of adrenaline ((12) and others) is slight or absent, nicotine causes definite constriction (12). This distinction we have confirmed. Further, on the coronary vessels, adrenaline acts as a dilator and nicotine as a constrictor (9). If these experiments on isolated tissues are valid their significance should be reflected on the pulmonary circulation in the intact animal.

Adrenaline in the cat always produces a profound rise in pulmonary

arterial pressure. This is the case even when the dose administered is so small as to cause a fall in systemic pressure; such a minute amount as 0·005 mg. causes a rise in pulmonary pressure nearly as large as with 0·02 mg. (Fig. 1). Adrenaline under no circumstances produces a diminution in the pulmonary pressure. Nicotine, on the other hand, has little effect on the pulmonary blood-pressure; when the dose is small no rise occurs, and it is only when the systemic pressure increases very considerably that the pulmonary pressure shows a rise; this rise is then never more than about 15 mm. of saline, and if such a rise occurs it is always associated with dilatation of the heart (Fig. 2) resulting from the large

Fig. 1.

Fig. 2.

Fig. 1. Cat. A.C.E. urethane. Vagi cut. Artificial respiration. Pulmonary and carotid blood-pressures. Adrenaline 0·005 and 0·02 mg. Rise in pulmonary pressure recorded in mm. half saturated sodium sulphate. (The rise in pulmonary pressure is nearly as marked with the smaller dose, when there is a fall in systemic blood-pressure.)

Fig. 2. Cat. A.C.E. urethane. Vagi cut. Artificial respiration. Pulmonary and carotid blood-pressures. Nicotine 0·05 and 0·1 mg. Rise in pulmonary pressure recorded as in Fig. 1. (No rise in pulmonary arterial pressure occurs with the smaller dose, and only a slight rise with the larger dose.)

increase in systemic blood-pressure. Occasionally after large doses of nicotine a transient increase in pulmonary pressure may be succeeded by a decided fall (Fig. 3) beginning after the maximum rise in systemic pressure has been passed. The explanation of these different effects with adrenaline and nicotine cannot be a question of back pressure, because an equal rise in blood-pressure with both drugs causes a very similar

degree of cardiac embarrassment. This we have shown on many occasions by experiments with the cardiometer and pressure records from the left auricle.

Adrenaline always produces an increase in the vascular volume of the lung and a considerable increase also in the volume pulse. Nicotine on the other hand causes at first a decided diminution in the vascular volume of the lung and of the volume pulse. The adrenaline effect is associated with a considerable increase in the right auricular pressure (Figs. 4 and 5); nicotine affects the right auricular pressure in a different

Fig. 3.

Fig. 4.

Fig. 3. Cat. A.C.E. urethane. Vagi cut. Artificial respiration. Pulmonary and carotid blood-pressures. Nicotine 0·143 mg. Changes in pulmonary pressure recorded as in Fig. 1.

Fig. 4. Cat. A.C.E. urethane. Vagi cut. Artificial respiration. Right auricular pressure, ung volume, and carotid blood-pressure. Adrenaline 0·01 mg. and nicotine 0·05 mg. Changes in right auricular pressure recorded in mm. half saturated sodium sulphate.

way according to the dose. Moderate amounts tend to increase it, though less than with corresponding doses of adrenaline (Fig. 4), and large amounts to increase it even more than with the largest doses of adrenaline (Fig. 5).

So that while adrenaline raises the pulmonary pressure and increases the vascular volume of the lung, nicotine causes relatively little or no rise in pulmonary pressure and diminishes the vascular volume of the lung. To obtain further evidence for the interpretation of these differences left auricular pressures were recorded in a series of experiments. This was done by inserting a long-necked glass cannula through a pulmonary vein

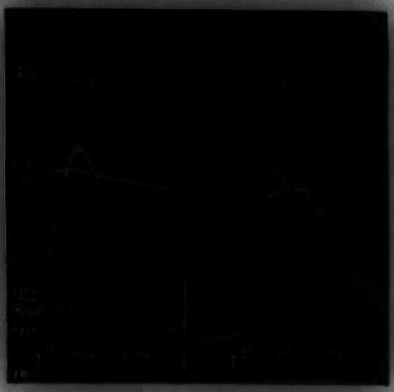

Fig. 5. Same experiment as Fig. 4. Adrenaline 0·04 mg. and nicotine 0·1 mg.

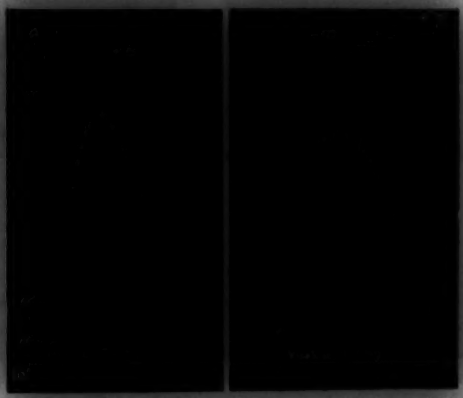

Fig. 6. Cat. A.C.E. urethane. Vagi cut. Artificial respiration. Left auricular and carotid blood-pressures. Adrenaline 0·04 mg. and nicotine 0·1 mg. Changes in left auricular pressure recorded in mm. half saturated sodium sulphate solution.

into the left auricle and recording the pressure changes with a manometer containing a half saturated solution of sodium sulphate. In such experiments, after all operative procedures were finished, hirudin was administered intravenously in order to minimise difficulties due to clotting. Injections of adrenaline in any dose under these conditions always produce a rise in left auricular pressure (Figs. 6, 7, 10). On the other hand,

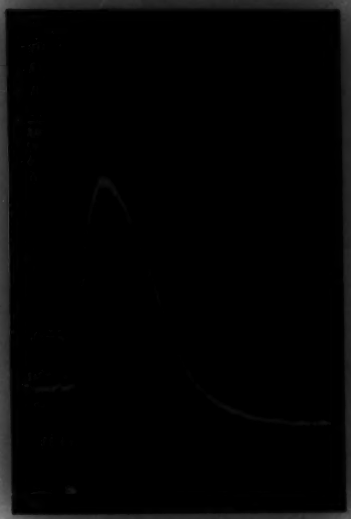

Fig. 7.

Fig. 8.

Fig. 7. Cat. A.C.E. urethane. Vagi cut. Artificial respiration. Right and left auricular pressures and carotid blood-pressure. Adrenaline 0·08 mg. Auricular pressures recorded as in Fig. 6.

Fig. 8. Same experiment as Fig. 7. Nicotine 0·2 mg.

injections of nicotine always produce a decided initial fall for a varying period up to a minute, followed by a rise (Fig. 6): in only one instance out of about twenty experiments have we seen an initial rise, and the effect here was clearly due to back pressure. If the right and left auricular pressures are recorded at the same time (Figs. 7 and 8) an injection of adrenaline causes the right auricular pressure to rise 5 seconds before the left auricular. A comparable dose of nicotine, injected in the same animal,

causes a fall in left auricular pressure 5 seconds before the rise in right auricular pressure.

The explanation of these facts may not be easy of interpretation, but we suggest that after small doses, which do not raise the blood-pressure much, adrenaline reaching the left ventricle would produce an increased flow of blood through the coronary vessels and in this way raise the minute volume return to the right heart and so increase the right auricular pressure. This effect would appear before an increase in left auricular pressure, since the latter must be dependent upon an increased flow of blood through the pulmonary system if no back pressure effects obtain. All changes following coronary dilatation will also come into play some few seconds earlier than those which follow cardiac embarrassment from systemic vaso-constriction.

Nicotine, on the other hand, causes definite constriction of the pulmonary vessels during the passage of the drug from the right to the left side of the heart, since the pulmonary pressure shows no fall and usually shows some rise, and the left auricular pressure diminishes. The rise in right auricular pressure, which occurs after large doses, appears about 5 seconds later, and must be regarded as the result of this pulmonary constriction, and perhaps also of back pressure due to cardiac embarrassment. Figs. 5 and 8 show that this secondary rise in right auricular pressure begins only when the left auricular pressure and lung vascular volume have begun to increase after the initial diminution, and when the pulmonary artery pressure rises: this suggests that here back pressure is the important factor. Fig. 9 shows the comparison between the rise in right auricular pressure due to high blood-pressure and that due to complete cardiac failure. The tracing was obtained during the injection of adrenaline into a cat, which caused the ventricles to fibrillate temporarily. The right auricular pressure rose about 15 mm. saline with the initial increase of blood-pressure, and the two curves ran roughly parallel. Immediately the ventricles fibrillated the right auricular pressure rose almost vertically to 40 mm. saline, and later when the ventricles spontaneously assumed a normal rhythm this enhanced back pressure effect disappeared. Fig. 10 shows a typical back pressure effect on the left auricular pressure due to cardiac embarrassment from systemic vaso-constriction.

Discussion.

These experiments demonstrate that adrenaline has no direct action of significance on the pulmonary blood vessels, and that the effect on the pulmonary circulation following its injection is due to other factors.

Adrenaline and nicotine have an identical type of action on systemic blood vessels; and also on the heart both directly and indirectly through systemic vaso-constriction. If the blood-pressure rises 50 mm. of Hg or more, the heart, with the pericardium open, dilates and the output diminishes, and this effect is associated with an increase in general venous

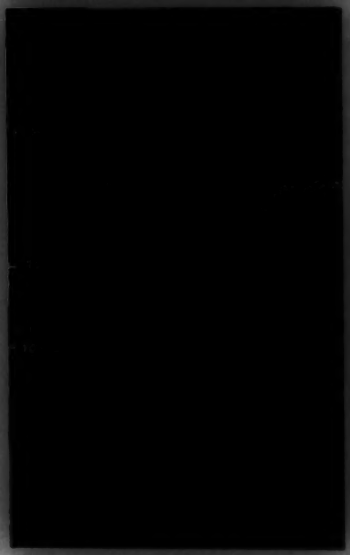

Fig. 9.

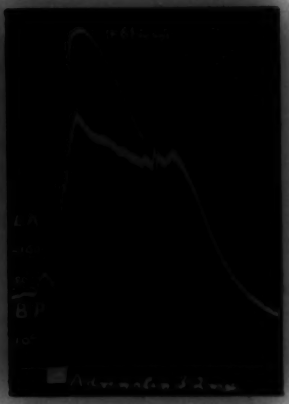

Fig. 10.

Fig. 9. Cat. A.C.E. urethane. Artificial respiration. Right auricular and carotid blood-pressures. Adrenaline 0·08 mg.

Fig. 10. Cat. A.C.E. urethane. Artificial respiration. Left auricular and carotid blood-pressures. Adrenaline 3·2 mg. The rise in left auricular pressure is recorded in mm. of half saturated sodium sulphate solution.

pressure and right auricular pressure. The rise in right auricular pressure is partly dependent upon the degree of cardiac dilatation which is associated with diminished output.

Adrenaline, even in small doses, dilates coronary vessels in the cat and dog and so increases the return of blood to the right heart. This we believe is sufficient to account for the increased pulmonary arterial

pressure in the intact animal when the rise in blood-pressure is small, just as it is in the heart-lung preparation ((5) and (17)). This view was confirmed by showing that adrenaline always augments the vascular lung-volume and nearly always increases the left auricular pressure. Very small doses of adrenaline which raise blood-pressure only a few mm. of Hg sometimes slightly diminish left auricular pressure, and this we believe to be due to the increased left ventricular output overshadowing the increased pulmonary inflow, since in such cases cardiometer experiments show that the cardiac output is increased.

Nicotine which undoubtedly constricts pulmonary vessels and to a smaller extent coronary vessels in perfusion experiments, raises the pulmonary arterial pressure very little, although the vascular volume of the lung and the left auricular pressure both diminish even with the smallest doses. That is the moderate pulmonary constriction has little effect in increasing pulmonary pressure. The fall in pulmonary pressure found in one experiment only (Fig. 3) we consider to have been due to an unusual degree of coronary constriction. It might be argued that the effects in all cases are due to diminished coronary flow which overshadows such pulmonary constriction as might be expected to occur from observations in perfusion experiments. If this be so, the diminution in vascular volume of the lung and the fall in left auricular pressure are not factors of pulmonary but of coronary constriction. It is improbable that this can be the correct interpretation because the right auricular pressure tends to rise. The coronary and pulmonary factors exert their effects in opposite directions; coronary constriction tends to lower the right auricular and pulmonary arterial pressures, and pulmonary constriction tends to raise them. When small doses are injected the coronary effect certainly influences the situation, though in experiments on isolated coronary vessels the degree of constriction with nicotine is small. When large doses are injected the right auricular pressure rises even more than it does with comparable doses of adrenaline, and at the same time the left auricular pressure and vascular volume of the lung decrease. These facts strongly suggest that in this case the pulmonary effect is predominant.

In the case of adrenaline the two factors work in the same direction, coronary dilatation and pulmonary constriction both tending to raise the right auricular and pulmonary pressures. In this case, however, as we have shown, the pulmonary factor is negligible.

Conclusions.

1. Adrenaline, injected intravenously into cats or dogs, causes a rise in pulmonary arterial pressure, an increase in the vascular volume of the lung, and increased outflow from the pulmonary veins, and a rise in the left auricular pressure. Direct action on the heart and coronary dilatation are considered to be the important factors in producing these effects.

2. Injections of nicotine have relatively little effect on the pulmonary pressure whilst diminishing the vascular volume of the lung, and causing a diminished outflow from the pulmonary veins, and a fall in the left auricular pressure. Constriction of the pulmonary vessels, and with very small doses the coronary, is considered to be the deciding factor responsible for the differences from the action of adrenaline.

3. Both drugs in large doses cause cardiac embarrassment from systemic vaso-constriction, and this results in secondary changes in the pulmonary circulation.

The work on which this paper is based has been concluded during the tenure by one of us (J. C. H.) of the Ernest Hart Memorial Scholarship of the British Medical Association.

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THE DISTRIBUTION OF THE BLOOD IN THE CORONARY BLOOD VESSELS.

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As a result of experiments on perfused dogs' hearts by Lovatt Evans and Starling(1) and on de Barenne's modification of the heart-lung preparation by Miyake(2), the conclusion was reached that the outflow of blood from the coronary sinus represents a constant fraction of the total coronary circulation. The authors considered that the average quantity of blood drained by the coronary sinus is 60 p.c. of the total coronary blood flow, the rest being drained by the small cardiac veins which open directly into the right auricle, and by the veins of Thebesius. The blood was collected from the sinus and from the right heart through the pulmonary artery in the isolated heart, and through the vena cava superior in the de Barenne's preparation. The blood flow through the veins of Thebesius which open into the left auricle and ventricle was therefore disregarded. The relative amount of blood flowing through the coronary sinus was found to be fairly constant for any given heart, and did not change in cases of vaso-dilation produced by asphyxia or by administration of adrenaline or in cases of increased perfusion pressure. Miyake adds to these observations that the relation between the outflow of blood from the sinus and the total coronary circulation remains approximately the same within fairly wide variations of the systemic output of the left ventricle and of the aortic pressure. It was only in cases of excessive output or pressure, when the dilation of the heart was such as to fill the pericardial sac, that the sinus outflow dropped to about 50 p.c. Opening of the pericardium restored the ratio to its previous value.

Recently Wearn(3) has published his observations on the human heart. The hearts were perfused with Ringer's fluid either a long time (48 hours) after death, in which case they failed to contract, or soon (2 to 4 hours) after death, the hearts beginning to beat spontaneously or under the influence of adrenaline. In the stationary heart the sinus outflow was a very small and variable fraction of the total flow, the bulk of the fluid escaping through the Thebesian vessels into the left and

right ventricles. In many experiments the sinus flow was only about 10 p.c. of the total. In the beating heart it constituted a somewhat larger proportion of the total flow, namely about 30 to 40 p.c. In experiments on the injection of the capillaries of the heart, Wearn finds that they become perfectly injected only in a beating heart, that they fill less completely in a non-distended heart, and scarcely at all in a dilated heart; in the latter case, the fluid escapes from the arteries through direct communications into the Thebesian system. It is suggested by Wearn that at some stage of its cycle the beating heart closes the Thebesian vessels and forces the blood through the capillaries into the sinus and other cardiac veins. The two experiments mentioned in Wearn's paper in which the coronary blood flow was measured in the same heart before and after the onset of contractions (hearts 41 and 53) fail to support this suggestion. After the onset of contractions the relative amount of the sinus outflow is only slightly increased:—from 29 to 32 p.c. in the first heart, and from 36 to 39 p.c. in the second.

The distribution of blood in the coronary veins. The large variations in the distribution of the coronary blood as indicated by Wearn's experiments made it necessary to verify the conclusions reached by the other authors, especially as the possibility of such variations throws grave doubt on the validity of all experiments in which the sinus outflow has been taken as a measure of the total coronary blood flow.

In view of Wearn's statement that the Thebesian blood flow into the left side of the heart may be considerable, we had to use a technique in which the whole coronary flow would be recorded without any loss of blood through unobserved channels. This could be best achieved by recording the inflow of blood into the coronary arteries rather than by measuring the various outflows as was done by previous investigators. The total inflow could then be compared with the outflow of blood from the coronary sinus, the difference being taken to represent the blood flow through the Thebesian vessels and the minor cardiac veins opening into the right auricle.

The experiments were performed on heart-lung preparations of the dog, and the method of measuring the inflow of blood into the coronary arteries was similar to that described by Anrep and Häusler(4). The coronary arteries were dissected at their origin, cannulæ were introduced into the right artery, and into the circumflex and the descendent branches of the left artery. The introduction of a cannula into the main stem of the left artery was found to be impracticable because its bifurcation is very often too close to the aorta, and sometimes even lies within the

aortic wall. After introduction of the three cannulæ, the small stretch of each artery between the cannula and the aorta was closely inspected to make sure that no branches of the arteries remained unperfused. In one experiment we found a small branch, but this was ligatured, and therefore we may say that in all our experiments the coronary circulation was entirely disconnected from the systemic circulation and was, so to speak, exteriorised. The experiments were not easy to perform: out of fourteen, only five were successful; however, once the perfusion actually started, the heart remained in excellent condition for several hours. The danger lies in ventricular fibrillation which developed in most of our failures during the introduction of the cannula into the descendent branch. The coronary sinus was drained by means of a Morawitz cannula. The inflow of blood into the arteries and the outflow from the coronary sinus were recorded continuously on smoked paper by means of small spirometers, which were calibrated to give an excursion of 2 cm. for a change of volume of 5 c.c. With blood flows of 30 to 70 c.c. per min. our measurements were accurate to less than half a cubic centimetre.

We shall first state the general conclusions which can be drawn from these experiments. It was found that the outflow from the coronary sinus bears an approximately constant relation to the total coronary circulation; if one takes into account all the measurements, this relation is not as constant as Lovatt Evans and Starling suggested, but in the majority of the experiments the variations are within such narrow limits that fairly accurate deductions can be drawn concerning the coronary blood flow from measurements of the outflow of blood from the sinus. Taking all the measurements on the beating, fibrillating or standstill heart, we find that the coronary sinus carries between 48 and 71 p.c. of the total coronary blood. The variations for any one heart are the same. Both extremes were observed only a few times, and under exceptional circumstances; in about 95 p.c. of our measurements the sinus blood flow was between 60 and 67 p.c. of the total, which agrees well with the observations of Lovatt Evans and Starling. The ratio was not much affected by changes of the systemic or perfusion pressures, by changes of the output or the temperature of the blood, or by application of vaso-dilator or vaso-constrictor substances. A few examples showing the extent of the effects are given in the following tables.

It can be seen from the first part of Table I that, at low aortic pressure, the absolute amount of blood flowing through the perfused coronary arteries, when the perfusion pressure is kept constant, is larger than at high pressure, which confirms previous observations of one of

TABLE I.

Heart-lung preparation. The temperatures of the systemic blood and of the coronary blood were 35·5° C. and 36° C. respectively, the heart rate varied between 130 and 135 beats per min.; the systemic output was 600 c.c. per min. The three coronary arteries were perfused at a pressure of 105 mm. of mercury. Each value for the blood flow is an average of five or more measurements, and only those observations were averaged in which the successive measurements differed by not more than 1 c.c. per min. Each new condition was maintained for not less than 5 min. The inflows of blood into the arteries and the outflows from the sinus are observed averages; the outflows from the rest of the veins are calculated.

(a) Effect of changes of the aortic blood-pressure.

Total coronary inflow in c.c. per min.	Outflow from coronary sinus in c.c. per min.	Unobserved outflow (Thebesian and minor cardiac veins) in c.c. per min.	Sinus flow expressed as percentage of total flow	Mean aortic blood-pressure in mm. of mercury
89	57·0	32·0	64·0	30
87	56·0	31·0	64·0	40
78	48·0	30·0	61·5	90
70	43·0	27·0	60·0	135
64	38·5	25·5	58·5	160

(b) Effect of changes of the perfusion blood-pressure; the aortic pressure is maintained at 80 mm. of mercury.

Total coronary inflow in c.c. per min.	Outflow from coronary sinus in c.c. per min.	Unobserved outflow (Thebesian and minor cardiac veins) in c.c. per min.	Sinus flow expressed as percentage of total flow	Perfusion pressure in mm. of mercury
34	24·0	10·0	71·0	30
55	37·0	18·0	67·0	60
84	53·0	31·0	63·0	130

us(4). With a change of aortic pressure from 160 mm. of mercury to 30 mm., the flow through the sinus is relatively more affected (increased by 48 p.c.) than the flow through the other channels (increased by 25·5 p.c.), in consequence of which at a low aortic pressure the ratio between the sinus and the total blood flow becomes slightly increased. The change in the distribution of the coronary blood indicates that, when the heart beat is strengthened by a rise in the aortic pressure, the compression of those blood vessels which emerge into the coronary sinus is somewhat greater than of those which constitute the Thebesian system. This is the usual behaviour of a heart which is in good condition, that is which does not dilate abnormally at a high aortic pressure. In a weak heart when the dilation is excessive, the ratio falls very considerably at a high aortic pressure, and this is independent of whether the pericardium is intact or not; and, as Miyake has pointed out, the sinus flow may be as low as 50 p.c. of the total or less.

The second part of the table shows that the effect of raising the perfusion pressure from 30 to 130 mm. of mercury is to diminish slightly the proportion of blood flowing through the sinus. The absolute blood flow is of course increased; but the outflow from the sinus is increased by 121 p.c., while the outflow from the other channels is increased by 210 p.c. This difference indicates that the increase of the perfusion pressure produces a smaller distension of those blood vessels which lead to the sinus than of those which have no connection with it. However, in most of our experiments, the effect was small. Table I is taken from an experiment in which the effect was more conspicuous than usual.

The effect of change of the systemic output is also very small, but the tendency is always for the ratio to diminish at large outputs. For instance, in one experiment an increase in the output from 600 to 1500 c.c. per min. changed the relative blood flow through the sinus from 62·5 to 60 p.c., and a subsequent diminution of the output to about 200 c.c. increased the sinus flow to 64·5 p.c. This observation, though of little importance in itself, adds to the value of experiments on the innervated heart-lung preparation (5) in which an increase of the output is accompanied by a considerable augmentation of the flow of blood through the sinus. We must, therefore, regard this latter effect as being of a genuine vasomotor origin, and not due to a change in the distribution of the blood in the coronary system.

Changes of the temperature of the systemic blood have no effect on the ratio; for instance, at a temperature of 30° C. the sinus flow was 67·0 p.c. of the total, and at a temperature of 39° C. it was 67·5 p.c. On changing the temperature of the perfusing blood the effect is very small, as can be seen from Table II.

TABLE II.

Heart-lung preparation. The systemic and the mean aortic pressures and the systemic output were kept constant. The temperature of the systemic blood was 38° C.

Total inflow in c.c. per min.	Outflow from the sinus in c.c. per min.	Unobserved flow through Thebesian and minor veins in c.c. per min.	Sinus flow expressed as percentage of total flow	Temperature of the perfusing blood in C.°	Heart rate per min.
65	43	22	66	37	155
73	45	28	62	29	145
83	50	33	60	27	140
63	42	21	67	36	—
59	40	19	68	39	160

With a change of temperature of the perfusing blood from 39° C. to 27° C. the blood flow through the sinus increased by 25 p.c., while the blood flow through the other channels increased by about 75 p.c.

Finally we must mention the effect of changes of the heart rate. This is best illustrated by a case of spontaneously developed partial heart block which was removed by an injection of a small dose of adrenaline into the perfused coronary arteries. At the time of the injection the heart was beating with a rhythm of 1 : 5 (auricles 150, ventricles 30). After the injection the rhythm changed through ratios of 1 : 4, 1 : 2, to a normal beat. The relative amount of blood flowing through the sinus was hardly altered: it was 62 p.c. during the heart block and 63 p.c. after its removal. The dose of adrenaline given was not large enough to produce an appreciable augmentation of the absolute blood flow, which was 63 c.c. before and 68 c.c. per min. after the injection. But even considerable changes in the tone of the coronary blood vessels have no obvious effect on the ratio, as was shown by Lovatt Evans and Starling for vaso-dilator substances, and as can be seen from the following experiment in which a severe coronary vaso-constriction was produced by injection of pituitary extract. In this case the inflow of blood was 63 c.c. and the outflow from the sinus 41 c.c. per min.; thus 65 p.c. of blood was drained by the sinus. After the injection of the extract into the coronary arteries the inflow diminished to 30 c.c., and the outflow to 20 c.c. per min., *i.e.* 67 p.c. of the blood was drained by the sinus. A further constriction of the coronary blood vessels led to a very irregular inflow and outflow of the blood.

The effect of ventricular fibrillation upon the distribution of the coronary blood is also negligible: for instance, in one experiment the outflow from the sinus was 65 p.c. of the total before and 63·5 p.c. during fibrillation; in another experiment the values were 64 and 62 p.c. respectively, and in a third 67 and 69 p.c. After some time the fibrillary movements usually become weaker and finally cease, but the relation between the sinus outflow and the total flow continues to remain constant within about 5 p.c. It is important, however, that in these experiments there should be no overfilling of the heart. Just as in excessive dilatation of the beating heart, an overfilling of the fibrillating or of the resting heart leads to a diminution of the relative amount of blood flowing through the sinus to about 50 p.c. or less. In the case of the fibrillating or resting heart, even a change in the position of the heart may affect the outflow of blood from the sinus: the weight of the heart pressing on the sinus, or the distended heart stretching it, may reduce the outflow

very considerably, without, however, much affecting the inflow into the coronary arteries. The best illustration of this is provided by experiments in which the coronary sinus is clamped. In one case the inflow was 60 c.c. while the outflow was 42 c.c. per min., or 70 p.c. of the total blood flow. The sinus was then clamped for 3 min.; the inflow of blood diminished within 30 sec. to 45 c.c. per min., and continued at that rate for as long as the sinus was clamped; on opening the sinus only a small amount of blood gushed out, and the inflow returned to 60 c.c. per min. During the clamping the cardiac veins were somewhat distended, but it is impossible to conceive that all the excess of blood flowing into the arteries was accommodated in the capillaries of the heart. Since the inflow was constant for 2·5 min., we must conclude that all the inflowing blood emerged from the heart through the minor cardiac veins and through the veins of Thebesius. During the period of clamping the blood flow through these channels therefore increased from 18 to 45 c.c. per min. Thus it is evident that these channels may serve as a very effective draining system for the coronary blood vessels when the blood-pressure in the coronary sinus is high. There is, however, no evidence to suggest that the contraction of the heart at some stage of its cycle forces the blood into the sinus, since the distribution of the blood is not altered by the heart ceasing to beat. It is the distension of the heart muscle and the consequent compression of the coronary sinus which prevent the blood from entering it, with the result that the blood becomes diverted into the Thebesian channels.

The distribution of blood in the coronary arteries. The method of perfusing the coronary arteries through three cannulæ enabled us to measure the amount of blood flowing through each of the arteries separately, and to estimate the relative amount of blood which each of the arteries supplies to the coronary sinus and to the remaining venous system of the heart. In one experiment the measurements were made after clamping any two of the three cannulæ so that the heart was for some time supplied only by a single artery. This method is satisfactory for the study of the amount of blood carried by each of the arteries, but it cannot be used for the determination of the distribution of the inflowing blood between the sinus and the remaining venous channels, because the clamping cannot be prolonged for more than about 30 sec., a length of time which is barely sufficient for the effect of clamping to be completely reflected on the outflow of blood from the coronary sinus. In the rest of the experiments therefore we always clamped only one artery at a time, and calculated the blood flow through this artery by difference. Clamping of one artery

could be prolonged for more than 1 min., which gave time for the sinus outflow to reach a steady level. Clamping of one or of two of the coronary arteries does not lead to an increased blood flow through the remaining artery or arteries. It must therefore be concluded that the time of clamping is insufficient for the collateral blood flow to develop to any appreciable extent. This can be seen from Table III.

TABLE III.

Heart-lung preparation. Two of the cannulæ perfusing the coronary arteries were clamped, and the inflow of blood into the remaining artery was measured. The duration of clamping was in every case about 1 min.

Blood pressure in mm. of mercury	110	90	60·0
Blood flow through the three arteries in c.c. per min.	86	69	40·0
" " the circumflex branch	41	35	18·5
" " the descendent branch	30	24	14·0
" " the right artery	14	11	6·0
The sum of the blood flows through the three arteries in c.c. per min.	85	70	38·5

It is evident that if the clamping of the arteries had led to an increased blood flow through the remaining artery, the sum of the blood flows through each single artery would be larger than the blood flow through the three arteries together. The agreement between the calculated and the observed total flow is so close that the possibility of a development of collateral circulation within the comparatively short duration of clamping is ruled out.

The relative amount of blood carried by any one of the three arteries is fairly constant. Taking all the observations into account it may be stated that the circumflex branch carries about 50 p.c. of the total coronary blood, and the descendent branch a little over 30 p.c.; that is, the left coronary arteries supply slightly over 80 p.c. of the blood to the heart, while the right artery supplies slightly less than 20 p.c. Examples of such measurements are given in Table IV. These readings were taken at random from different experiments with different perfusion pressures.

TABLE IV.

Total inflow in c.c. per min.	Blood flow in the coronary arteries expressed as percentages of the total inflow			
	Circumflex artery (C)	Descendent artery (D)	Left coronary artery ($C + D$)	Right coronary artery
111	48	33	81	19
81	52	34	86	14
63	50	28	78	22
40	48	35	83	17

In one experiment the descendent branch was very small and, under various conditions, it carried only about 20 p.c. of the total coronary

blood, but the circumflex artery was abnormally developed and carried about 60 p.c. of the blood, so that in this case also 80 p.c. of the blood passed along the left coronary artery.

Each of the arteries supplies some blood to the coronary sinus and to the other channels, amongst which are the Thebesian veins. Knowing the blood flow through an artery, and the extent to which the sinus flow is affected by clamping this artery, it is easy to determine the distribution of the blood carried by it. A few examples of such determinations are given in Table V.

TABLE V.

The circumflex and the descendent arteries and the right coronary artery are designated *C*, *D* and *R* respectively.

Inflow of blood into the coronary arteries in c.c. per min.			Blood supplied by each artery to the coronary sinus in c.c. per min.			Blood supplied by each artery to the remaining channels in c.c. per min.			Percentage of blood supplied by each artery to the coronary sinus		
C	*D*	*R*	*C*	*D*	*R*	*C*	*D*	*R*	*C*	*D*	*R*
23	14	9	15·5	9·0	3·5	7·5	5·0	5·5	67	65	39
32	19	12	24·0	12·0	4·0	8·0	7·0	8·0	75	64	33
44	28	20	31·0	18·0	8·0	13·0	10·0	12·0	70	64	40

As the result of these and similar experiments it can be estimated that over 60 p.c. of blood flowing through the right coronary artery fails to enter the coronary sinus, while in the case of the left artery only 30 to 35 p.c. misses the coronary sinus.

We believe that the experiments described in this paper fail to support the conclusions reached by Wearn, in so far as his conclusions are concerned with the difference in the distribution of the coronary blood between the coronary sinus and the other venous channels, in the beating and standstill heart. The suggestion that the heart at some stage of the cycle occludes the Thebesian blood vessels and forces the blood into the coronary sinus is not supported by the experiments described here.

CONCLUSIONS.

1. The conclusion reached by Lovatt Evans and Starling that about 60 p.c. of the coronary blood escapes through the coronary sinus is confirmed.

2. This relation between the outflow of blood from the coronary sinus and the total coronary circulation is only slightly affected by changes of the aortic pressure, output of the heart, pressure perfusing the coronary arteries, heart rate and temperature of the blood.

3. The distribution of the coronary blood flow between the sinus and the other channels is the same in the beating, fibrillating or standstill heart.

4. In cases of considerable rise of intraventricular pressure due to overfilling of the ventricles, less blood flows through the coronary sinus and more through the other venous channels of the heart; this also occurs when the pressure in the coronary sinus is increased.

5. The relative distribution of the flow between the coronary arteries, and the distribution of the blood carried by each of the arteries between the coronary sinus and the other venous channels are shown to be constant within narrow limits.

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CROSSED EXTENSOR REFLEXES AND THEIR INTERACTION.

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I. Introduction.

Whereas the "principle of convergence"(21) of different spinal paths upon the motoneurones forming their "final common path" has been repeatedly studied on "antagonistic" reflexes ((23), p. 135), the same problem has received much less attention from the closely related aspect of "allied" reflexes ((23), p. 120) influencing each other. Earlier investigations by Sherrington(22) and Camis(5) were recently extended by Cooper, Denny-Brown and Sherrington(8), who, using a method similar to the one employed in this paper, and working with ipsilateral spinal flexor reflexes, analysed in detail the types of interaction met with. When the individual reflexes from any nerve pair were elicited in such a temporal combination that they overlapped, it was usually found that the resulting reflex contraction in the muscle investigated was less than the algebraic summation of the two reflex responses taken individually. The deficiency was shown to be due to a process which was designated occlusion, and which is really a manifestation of the "principle of convergence." Sometimes definite evidence of inhibition was met with, and, on comparatively rare occasions, one reflex seemed to be facilitated by the other. The full relevance of these conceptions will be explained later.

On account of the differences shown(17, 18, 19) to exist between flexor and crossed extensor reflexes, it was considered important to carry out an investigation similar to the above, on the crossed extensor reflex.

II. Method.

The animal (cat) being deeply anæsthetised, deafferentation was performed; the 4th, 5th and 6th post-thoracic roots for quadriceps and the 6th, 7th, 8th and 9th for soleus and gastrocnemius(20). Decerebration (intercollicular) by the trephine method immediately followed. The anæsthetic was discontinued and the rest of the operation including the immobilisation, by appropriate nerve and tendon section, of every muscle

except the one to be recorded from, and the denervation of the skin, was continued after an interval.

At first the whole quadriceps was recorded from, but for the most part only one or two of its component parts have been used because it was suspected that its component muscles did not behave in identical fashion, and this suspicion was later confirmed. The buried electrodes (9) were found successful in avoiding deterioration of the nerve stimulated.

Deafferentation, though adding to the severity of the operation, was thought necessary, to guard against excitatory and inhibitory influence ((26), (16), (15) p. 436, and (11)) arising from the proprioceptors of the contracting muscle itself.

Fixation for quadriceps has been secured by steel drills in both ends of the femur and a pelvic clamp, all of which have been rigidly attached to uprights clamped to the heavily weighted table on which the preparation rests. For soleus it is necessary to drill the tibia at both ends, and for gastrocnemius the femur in addition has to be similarly fixated. Care has been taken to give the muscle free play by isolating it from surrounding fascia and muscles, and in a groove in the piece of bone which has been cut out at its insertion, rests the steel hook which connects it to the lever arm of the high frequency isometric optical myograph which has been used throughout this investigation. The more recent work has been done with the improved mirror myograph (10) which has enabled us to record from two muscles simultaneously. Especial care has then to be taken to adjust the initial tension of each muscle to a comparable amount and a complete separation of the two muscles is essential.

Time has been recorded by a Rayleigh magnetic wheel. Stimulation has been by coreless Berne coils delivering about 50 double shocks per sec. with two volts in the primary. (With the double myograph 90 double shocks per sec. were used.) The signals have been worked by the camera and the shadow of the short-circuiting key of the secondary has been thrown on the camera slit by the same source of light as was used for the myograph.

Owing to the influence of the labyrinthine and neck reflexes (1) on the reflex centres of the cord, care has been taken to keep the head in a fixed position during a series of observations. It has generally been in "minimal" position and the animal has rested on the side opposite to the muscle which is being recorded from. The temperature has been taken deep in the inguinal wound at intervals during the experiments, and, as a rule, has been kept fairly steady about 36° C. Variations of 2 degrees from this were rare.

As a rule no observations have been made until at least 4 hours have elapsed after the cessation of the anæsthetic. Even then it has generally been observed that the crossed extensor response has increased steadily during the experiment even for as long as 10 hours after the operation.

When studying interaction we have usually combined the two reflexes in question in overlapping or intercurrent sequence, but sometimes both have commenced almost simultaneously. Control observations of each individual reflex are important as inconstancy is frequent. All unreliable observations have been discarded. The agreement of controls was often not so absolute as is shown in the diagrams, but in these and other cases from which data have been derived, the variations are so slight as to be

negligible. All the figures except Fig. 1 are tracings from the negatives of the photographic records.

III. Results.

1. *Crossed extensor reflexes.*

The main features of this type of reflex (23), (26) and its reciprocal relationship (24), (27), (28) have already been described. Graham Brown(3) has given an account of the crossed extensor reflex and its numerous variations as it is seen in the gastrocnemius-soleus muscle. More recently Liddell and Sherrington (17), (18), (19) have described in detail the differences existing between the crossed extensor reflex and the flexor reflex. In the former temporal summation plays an important part revealed by progressive involvement of fresh motor units, "recruitment," and after-discharge is a marked feature in the undeafferented preparation(26). Fulton and Liddell(16) have produced evidence showing that recruitment was to a large extent produced by "autogenous" inhibition arising from the muscle's own contraction. Denny-Brown(11) has proved the presence of this inhibition.

Gastrocnemius has been an unsatisfactory muscle as its response even to strong stimulation of crossed nerves is usually very small. In this we confirm Denny-Brown(12). We will confine our attention to soleus and various component muscles of quadriceps.

Latent period has been of very variable duration. The individual makes and breaks of the primary were not photographed, so that, as the unshortcircuiting signal has to be used as the basis of measurement, an error of several sigmata was always possible. This error was often minimised by taking the shortest latency of a series. In the deafferented quadriceps the latent period is, as a rule, longer than in the undeafferented quadriceps in which latencies shorter than 40σ were common, and in one case a latency of 22σ was observed. 40σ has been the shortest latency which we have recorded in the deafferented preparation, but values between 40 and 50σ have been common. With soleus muscle latencies have been somewhat longer and values less than 60σ have been rare. It has been impossible to establish any regular relationship between the length of the latent period and the nerve stimulated. Weakening the reflex has invariably lengthened the latent period(18), and latencies up to 500σ have been observed.

Recruitment. As a rule in the deafferented preparation the long gradual rise described by Liddell and Sherrington(18) for the undeafferented preparation has been a rare feature, and at times the reflexes

have been almost "d'emblée" in character. Where control motor tetani have been elicited by stimulation of the motor nerve we may obtain some idea of how closely the crossed extensor reflex sometimes approximates to the motor tetanus. As a standard of measurement in any particular case we have used the time from the first sign of contraction to the attainment of 90 p.c. of the total tension developed in that case.

In the experiment quoted below all the stimulations were maximal and, in all, the reflex responses were at least 50 p.c. of the motor tetanus. Both muscles were recorded from simultaneously.

Muscle	Motor tetanus	Peroneal* nerve	Post-tibial* nerve	Quadriceps* nerve
Vastus medialis	160σ	230σ	200σ	260σ
Vastus lateralis	120σ	230σ	210σ	280σ

* Only the effects of stimulating nerves of the hind limb opposite to that of the registering muscle are considered in this paper.

In these cases the total period of recruitment must be practically over in 200σ and the greater part of it has occurred within a period of 100σ from the earliest reflex response. When peroneal and post-tibial nerves were stimulated simultaneously, vastus medialis developed 90 p.c. of the total tension in 170σ. In this case the reflex discharge of all the motoneurones was almost simultaneous. In Fig. 7 are further examples of rapid recruitment.

Soleus generally shows a more gradual recruitment.

This is shown in Fig. 6 *C*, though the soleus in this case is particularly rapid, for a motor tetanus reaches 90 p.c. of its total tension in 195σ (260 to 290σ are more normal figures in our experience). The maximum reflex responses to internal saphenous and post-tibial nerves take 420 and 480σ respectively. With weaker reflexes there is but little increase in this period (Fig. 6 *A* and *B*).

Contrary to the finding of Fulton and Liddell(16) we have occasionally found the two-stepped ascent in the deafferented preparation. In these cases, however, there was always other evidence, especially rebound, of the presence of inhibition.

After-discharge. With deafferented quadriceps after-discharge at plateau height usually continued for 100 to 200σ for reflexes of 1 to 1½ sec. duration and then the tension dropped abruptly. With motor tetani of similar duration the time from the cessation of the stimulus to the angle was 35 to 40σ. In those cases where the reflex was falling away before the cessation of the stimulus a more abrupt falling away commenced often at much less than 100σ. With soleus where the terminal mechanical response of the motor tetanus has been about 80 to 90σ, the after-discharge is also usually longer, often much greater than 200σ.

When the preparation has been in bad condition there has been an enormous prolongation of after-discharge, the return to resting tension often taking as long as a minute. This is not a contracture due to fatigue of the peripheral mechanism (2), for motor tetani were normal.

Fractionation of quadriceps. With a double myograph vastus medialis and vastus lateralis have been used together. There was a striking similarity in the responses of the two muscles (Fig. 9), though closer analysis has revealed a small but constant difference between them. Fig. 1, where the maximum reflex tetanic responses of both, simultaneously

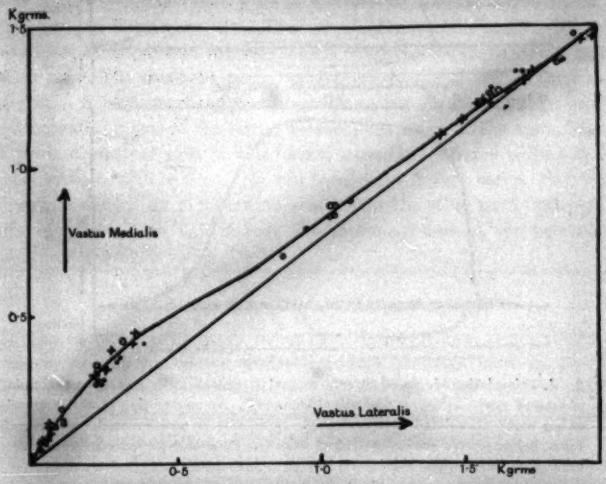

Fig. 1. Graph of simultaneous tension development in vastus medialis (ordinates) and vastus lateralis (abscissæ) (both deafferented). Crosses responses to superficial peroneal nerve, dots to post-tibial nerve, and circles to stimulation of both simultaneously.

recorded, are plotted against each other, shows the remarkable constancy of their relative contractions. Strong reflexes were interspersed with the weak so that no gradual change the preparation was undergoing would affect the results. Though some intermediate values are missing it is obvious that with weaker reflexes vastus medialis tends to predominate, but near threshold it falls off more rapidly than does vastus lateralis so that their thresholds were the same. The responses to post-tibial nerve

and to superficial peroneal nerve lie on the same curve. The predominating response of vastus lateralis in strong reflexes is attributable to its greater size and not to a greater proportion of its motor units being excited.

Rectus femoris and vastus medialis as a pair show less similarity (Fig. 2). Threshold has been distinctly lower for vastus medialis, and

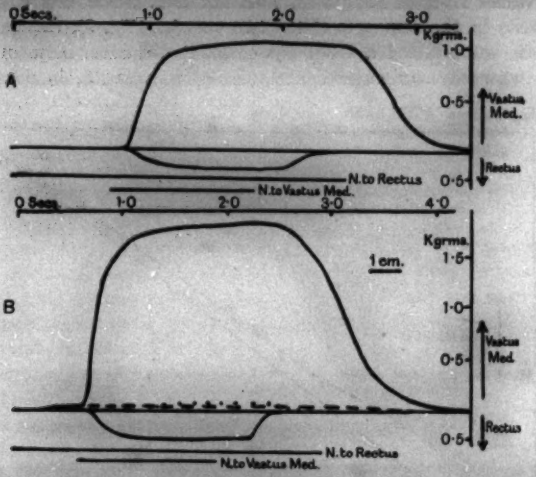

Fig. 2. *A*. Stimulation of crossed nerve to rectus (12 cm. coil) with intercurrent stimulation of crossed nerve to vastus medialis (14 cm. coil). No contraction of either muscle to either nerve stimulated independently. Both muscles deafferented. Magnif. = 550. Lines below curves show duration of stimuli. Time is recorded on line above and tension on line to right side.

B. As above but strengths stimulation increased to 11 cm. coil for nerve to rectus and 13 cm. coil for nerve to vastus medialis. Each alone causes a small contraction in vastus medialis as is shown by dashes for nerve to rectus and by dots for nerve to vastus medialis. Rectus muscle still does not respond to either nerve stimulated independently.

with weak reflexes the latent period has been longer for rectus, but for strong reflexes the latent periods of both muscles have been approximately equal. In rectus there has been more abrupt tension development, plateau after-discharge is shorter and the decline of tension much more rapid. Sustained facilitation (see later) though present is much less prominent in the reactions of rectus than it is with vastus medialis (Fig. 2). Decline of response of rectus has occurred simultaneously with

increase in vastus medialis. Marked rebound has been observable in rectus with none, or only a trace, in vastus medialis. Sometimes in the interaction of two reflexes occlusion has occurred in vastus medialis simultaneously with facilitation in rectus. Such findings as these emphasise the importance of recording from only one component of quadriceps at a time.

There is, therefore, a certain selective excitation of the motoneurones of the whole quadriceps centre according to which component muscle they belong. With vastus medialis and vastus lateralis this is slight, and it is highly probable that a large number of afferent nerve fibres are "proper" to both muscles (7). The remarkable relative constancy of the responses of these two muscles seems incapable of explanation in any other way. The much more distinctive behaviour of the motoneurones of rectus is not surprising, because rectus, being a double joint muscle, is a potential flexor of the hip as well as an extensor of the knee. Though it probably never acts in the former capacity, reflexes calling it into activity have also to fixate the hip to prevent flexion there. One would therefore expect its motoneurones to be for the most part "proper" to afferents which do not include the motoneurones of the rest of the quadriceps in their reflex "field."

2. *Interaction of crossed extensor reflexes.*

The features evident in interaction between two crossed extensor reflexes can be considered under the same headings used for the ipsilateral spinal flexor reflexes (8).

(i) Two reflexes when combined produce more tension in the muscle than summation of their individual effects—facilitation.

(ii) Two reflexes with little or no evidence of possessing inhibitory components give, when combined, less tension development in the muscle than summation of the results of each singly—occlusion.

(iii) More complex phenomena exhibited by reflexes showing marked inhibitory components.

The tension developed by the muscle has been used as a measure of number of motor units excited. Denny-Brown (12) has shown this to be approximately correct.

(i) *Facilitation.*

A. In *concurrent facilitation* the effect is exerted by one reflex on another simultaneously excited. We have found that concurrent facilitation is more easily and regularly obtained in the crossed extensor reflex

than was found to be the case with the flexor reflex (8), but it has been rather rare to find that two reflexes, themselves subliminal, are capable of evoking a marked reflex response when concurrent.

Thus, neither post-tibial nerve nor superficial peroneal nerve caused any response in crossed deafferented soleus when stimulated independently, but when concurrent evoked reflex contractions of 90 and 110 grm. on two successive occasions. The crossed nerve to vastus medialis, and the crossed nerve to rectus, stimulated independently, repeatedly produced no reflex response. When stimulated concurrently a large contraction in both rectus femoris and vastus medialis was regularly produced, *e.g.* 1·1 kg. in vastus medialis and 0·35 kg. in rectus (Fig. 2 *A*).

Somewhat similarly when the stimulation of one nerve alone is inefficient, when intercurrent it facilitates a weak reflex from another nerve (Fig. 3 *B*), or, inversely, if the weak reflex is intercurrent in

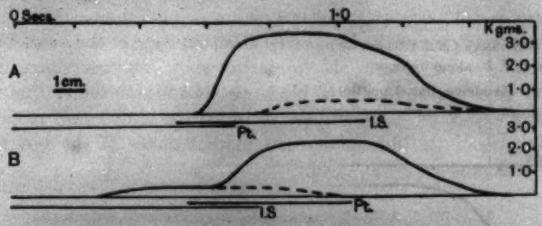

Fig. 3. *A*. Stimulation of crossed popliteal (*Pt.*) and internal saphenous (*I.S.*) nerves in overlapping sequence. Popliteal alone (16 cm. coil) was just below threshold. Internal saphenous alone (15·5 cm. coil) produced a small response as shown by dotted line. Muscle-rectus with vastus lateralis (deafferented).

B. As above, but stimulations overlap in reverse sequence.

the non-effective stimulation, the weak reflex is markedly facilitated (Fig. 3 *A*).

Thus crossed nerve to vastus medialis caused a small reflex response of about 50 grm. in the vastus medialis muscle and nothing in rectus. Independently the crossed nerve to rectus had no reflex effect on either muscle, but, when intercurrent in the weak reflex from nerve to vastus medialis, it caused an abrupt tension development of 2·5 kg. in vastus medialis and 1·4 kg. in rectus.

Facilitation has been commonest between weak reflexes.

Thus (Fig. 6 *A*) soleus develops 320 grm. with post-tibial nerve alone and 480 grm. with internal saphenous nerve. When both are stimulated together 950 grm. tension is produced. Again with soleus 70 grm. from superficial peroneal nerve and 180 grm. from post-tibial give 300 grm. when concurrent. With vastus medialis 0·40 kg. from small sciatic alone, and 1·25 kg. from one plantar facilitate to give 2·37 kg. when combined, and again with vastus medialis. (Fig. 4 *A*) 0·16 kg. from one plantar and 0·22 kg. from the other give 0·63 kg. when combined.

Facilitation is particularly frequent and sometimes extreme between afferent nerve pairs closely allied in distribution, *e.g.* two nerve branches to quadriceps, or two plantar nerves.

Facilitation invariably shortens the latency of the response to the

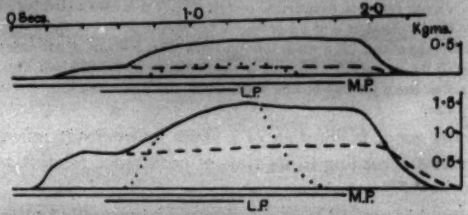

Fig. 4. *A*. Lateral plantar nerve stimulus (*L.P.*, 30 cm. coil) intercurrent in medial plantar nerve (*M.P.*, 28 cm. coil). Contraction to *M.P.* alone is shown by dashes, and to *L.P.* alone by dots.

B. As above, but strengths of both lateral and medial plantar stimuli increased to 26 cm. coil distance. Deafferented vastus medialis. Magnif. = 550.

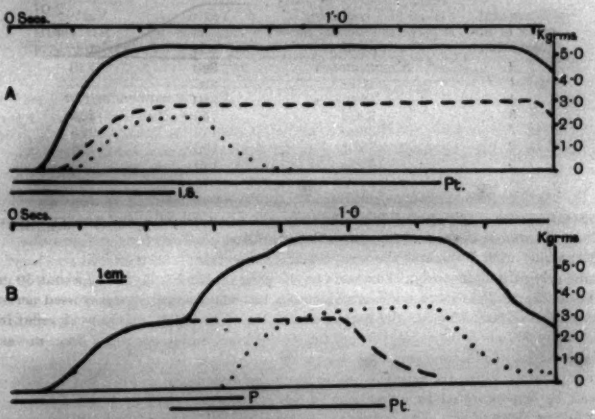

Fig. 5. *A*. Deafferented vastus lateralis and rectus, stimuli to internal saphenous nerve (*I.S.*, coil 10 cm.) and popliteal nerve (*Pt.*, coil 14 cm.). Control reflexes: dashes for *Pt.* and dots for *I.S.*

B. Same muscle and preparation as above. Stimulations applied to peroneal nerve (*P.*, coil 13 cm.) and popliteal (*Pt.*, coil 16 cm.). Dashes show peroneal reflex alone and dots popliteal reflex alone.

intercurrent stimulus, or, if both stimuli commence almost simultaneously, the combined response commences earlier than with either individually.

Thus (Fig. 4 *A*) the latent period of the intercurrent plantar is 220σ when alone and 70σ when intercurrent. Fig. 2 *B* is an extreme example as the latency of the response to stimulation of the nerve to vastus medialis is 400σ when alone and only 40σ when intercurrent in a weak response to nerve to rectus. Fig. 3 *A* is also a striking example. In Fig. 8 *A* where the facilitation is of a less degree the latent period of the small sciatic response is 170σ when alone and 135σ when intercurrent. Fig. 5 *A* and *B* are marked examples of shortening of latent period with only a small degree of facilitation. Thus (Fig. 5 *B*) the popliteal reflex has a latency of 94σ when alone and 44σ when intercurrent in a peroneal nerve response.

The degree of facilitation of a short intercurrent reflex of constant length varies according to its time of occurrence relative to a constant long continued reflex.

Thus superficial peroneal nerve was stimulated intercurrently for 0·58 sec. at various periods relative to a post-tibial nerve stimulation lasting 2·34 sec. The two muscles registered simultaneously and the maximum tensions developed are tabulated below. Both nerves were in fluid electrodes and the strength of stimulation was at 16½ cm. coil distance which was just above threshold, a condition necessary for the occurrence of facilitation.

Temporal order of observation	Interval between commencement of post-tibial and beginning of superficial peroneal nerve stimulation	Vastus medialis tension in grm.	Vastus lateralis tension in grm.
7	Simultaneous	340	230
1	100σ	420	320
4	160σ	280	230
5	230σ	150	115
6	360σ	150	115
2	760σ	190	120
3	1580σ	130	90

During this series of observations repeated controls were taken of the responses elicited from each nerve singly, and a fair constancy was observed with, however, a noticeable falling off towards the end of the series. This doubtless accounts for the high value of the observations with 760σ and the comparatively low value obtained with both reflexes commencing simultaneously. The average values for superficial peroneal nerve alone were 100 and 75 grm. for vastus medialis and vastus lateralis respectively, and for post-tibial, nerve alone 55 and 40 grm. The background reflex (post-tibial) definitely showed falling away after 600σ and the explanation of the lessened facilitation occurring when the superficial peroneal was intercurrent after this period is certainly due to this so-called "fatigue" of early onset. What its nature is we do not know (*vide infra*, p. 116), but it seems that it can be demonstrated by a lessening of the facilitating power of a reflex long before any falling away of the already existent contraction is apparent, *e.g.* facilitation at 230σ interval is definitely less than at 160σ though falling away was not evident till 600σ.

This curious diminution of facilitating power in the latter parts of a reflex response was also shown by the effect of an intercurrent break shock to a crossed nerve. Alone a shock to internal saphenous nerve produced a 20 grm. contraction in soleus, but, when intercurrent in a reflex from post-tibial 0·77 sec. from its commencement (plateau had just been reached), there was a tension increase from 340 to 510 grm. When the same shock was 1·75 sec.

from the commencement of the background reflex, then the tension increase was only from 320 to 360 grm. This reflex background had shown no signs of falling away and yet this marked diminution of its facilitating power had occurred.

In Fig. 6 *B* a very early intercurrent stimulus produces less extra tension rise than one occurring later. Similar results have also been

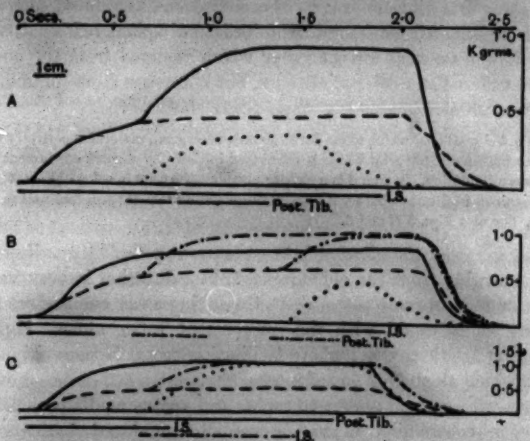

Fig. 6. *A*. Deafferented soleus post-tibial nerve (coil 20 cm.) intercurrent in an internal saphenous nerve stimulation (coil 33 cm.). Dashes show internal saphenous alone, and dots post-tibial alone.

B. Muscle and nerves as above and same strengths stimuli to both. As shown by lower signal lines post-tibial stimulus incident at various points in reflex background of internal saphenous. Continuous line reflex corresponds to the continuous line signal for post-tibial nerve. The dot dash line corresponds to the dot dash signal and the double dot dash line to the double dot dash signal. Corresponding to this latter is control of post-tibial reflex alone. Dashes show the control of internal saphenous alone.

C. Muscle as above. Post-tibial at 16 cm. coil with internal saphenous at 20 cm. coil intercurrent at two different times. The dashed line is post-tibial reflex alone, and dotted line internal saphenous reflex alone.

obtained with early intercurrent break shocks. They are most often observed with the comparatively slow recruiting reflexes of soleus as backgrounds. It seems that the facilitating power of a reflex is not maximal until recruitment is almost complete. These two factors of "fatigue" and recruitment are opposed to each other, and the point of

optimal facilitation seems to be after recruitment is practically complete and before "fatigue" has advanced very far.

B. In *sustained facilitation* the facilitation has appeared as an after-effect of one reflex on the other. This has been a very constant and striking feature in our investigations on the crossed extensor reflex (both when the interacting reflexes have occluded and facilitated each other). This is in marked contrast to the ipsilateral spinal reflex ((8) p. 291) where it was unusual for the reflex which emerged from the occlusion to have suffered any marked changes, but it appears more common when the intercurrent stimulus is a single break shock (13).

In Fig. 5 *A* popliteal, which alone develops only 2800 grm., maintains perfectly for 1 sec. a plateau height of 6100 grm. which is developed initially by a short concurrent internal saphenous stimulation. In Fig. 6 is a similar phenomenon seen in soleus. Figs. 4 *A* and *B* show sustained facilitation both with occlusion of strong reflexes and facilitation of weak reflexes. See also Figs. 5 *B* and 8.

How long can the effects of the concurrent reflex be manifest in the reflex emerging from the concurrence? The duration is very variable, as can be seen in some cases where the falling away commences almost as early as it would with the intercurrent reflex alone. Especially interesting are those cases where facilitation occurs between two stimulations which alone give no reflex response.

Thus with crossed nerves to rectus and vastus medialis as afferents the reflex contraction in vastus medialis produced by concurrent facilitation is maintained by nerve to rectus alone for 630σ. However there is less "central drive," for after-discharge (at plateau height) is then only 160σ when stimulation of nerve to rectus ceases, whereas when stimulation of nerve to vastus medialis ceases only 75σ before that of nerve to rectus, the after-discharge at plateau height is 260σ. With longer continuance of stimulation of nerve to rectus after concurrence it was found that tension only fell from 0·68 kg. to 0·62 kg. in 1·6 sec., and it took 4·8 sec. for the sustained facilitation to completely disappear.

Again, with soleus (deafferented) the concurrent stimulations to post-tibial nerve and to internal saphenous nerve developed a plateau tension of 1·05 kg. On cessation of the stimulus to internal saphenous, which alone developed 0·90 kg., there was no fall in plateau height for 1·6 sec., and, when the stimulus of post-tibial nerve was finally discontinued 1·82 sec. later, the tension had only fallen to 0·77 kg., though post-tibial nerve alone was only capable of developing a tension of 0·66 kg. Here a considerable facilitation had persisted for 3·42 sec.

In the crossed extensor reflex sustained facilitation has been demonstrable with all nerve pairs and muscles used, exceptions being of course complete occlusion of the precurrent or intercurrent reflex and also when

one reflex markedly inhibits the other. From the foregoing account it is obvious that the phenomenon is allied to after-discharge. The only other possible suggestion would be an effect of the nature of rebound due to some underlying inhibition. In the cases discussed there has, however, been no evidence whatever of any marked inhibitory factor. Moreover, it is hardly likely that rebound should exactly and precisely equal the amount of the additional contraction due to the concurrence.

(ii) *Occlusion.*

We have found occlusion between reflexes excited from even the most diverse nerve pairs. Its amount has been estimated by observing the percentage deficiency of a reflex contraction when intercurrent, taking as standard the reflex contraction elicited alone. It is recognised that this is a purely arbitrary value as it assumes that all the occlusion has occurred at the expense of the intercurrent reflex, a totally different value being obtained if the reflex sequence is reversed (8). No account is taken of simultaneous facilitation which doubtless often occurs, and which would tend to make the amount of occlusion appear smaller than it actually is.

In agreement with findings on the ipsilateral spinal flexion reflex (8) we have found in the crossed extensor reflex:

1. Those afferent nerves which produce the largest reflex responses are as a rule the most powerful occluders. Thus popliteal or post-tibial nerves which evoke large reflex responses in quadriceps, or its component muscles, are powerful occluders.

2. As the individual reflex responses from any particular nerve pair increase (due to increase in strength of stimulus), the degree of occlusion between them increases.

3. The degree of occlusion decreases in those cases where the reflex background falls away.

4. Other things equal, the degree of occlusion is greater between afferents having a closely allied peripheral distribution, *e.g.* plantar nerves.

Our findings for the crossed extensor reflex have, however, differed from those for the ipsilateral flexor reflex (8) in that we have found that the amount of occlusion was dependent on the temporal position of the intercurrent reflex relative to the background reflex.

Thus in Fig. 7 *A* when the internal saphenous response is intercurrent in the post-tibial response it is practically totally occluded. When both stimulations commence together there is much less occlusion (Fig. 7 *B*), and this lessened degree of occlusion is maintained throughout the reflex. Again for vasto-crureus, a peroneal stimulus when intercurrent was

completely occluded by popliteal, but, when both commenced together, there was only 70 p.c. occlusion.

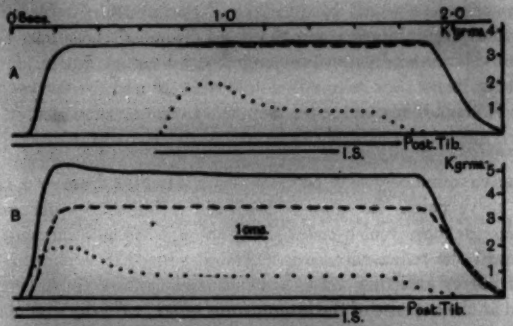

Fig. 7. *A*. Deafferented vastus lateralis. Internal saphenous nerve stimulation (coil 10 cm.) intercurrent in post-tibial nerve stimulation (coil 13 cm.). Dotted line shows control of internal saphenous alone, and dashes post-tibial alone.

B. As above but internal saphenous stimulation begins simultaneously with post-tibial. Controls shown as above.

A similar feature has already been described when dealing with facilitation and it seems likely that in this case the variation in amount of occlusion is only apparent, the real cause being the increased facilitation simultaneously occurring when the intercurrent reflex is early.

The latent period of the increase in tension produced by an intercurrent reflex has not borne a constant relation to the latent period of that reflex when elicited independently. Thus in Fig. 4 *B* the latent period of the lateral plantar undergoes no detectable change when it is intercurrent, both latencies being about 80σ. Again the latent period of peroneal is lengthened from 48σ when alone to 136σ when following on a popliteal reflex. Again in Fig. 8 *B* the latency of the response to small sciatic is increased from its independent value of 90σ to 170σ when it is intercurrent. This case is particularly interesting because when both stimuli were weak a shortening of latent period had occurred (Fig. 8 *A*), as already mentioned under facilitation. It seems that two different processes are involved. In Fig. 8 *A* there is an earlier response to the small sciatic stimulus when intercurrent, because certain neurones have been subliminally excited by the plantar nerve stimulus and so are partly prepared to respond to the small sciatic. In Fig. 8 *B* where almost total occlusion occurs this facilitation is not present, and, moreover, all the

low threshold neurones of small sciatic which would soon recruit over the threshold are already excited. The latent period is therefore necessarily

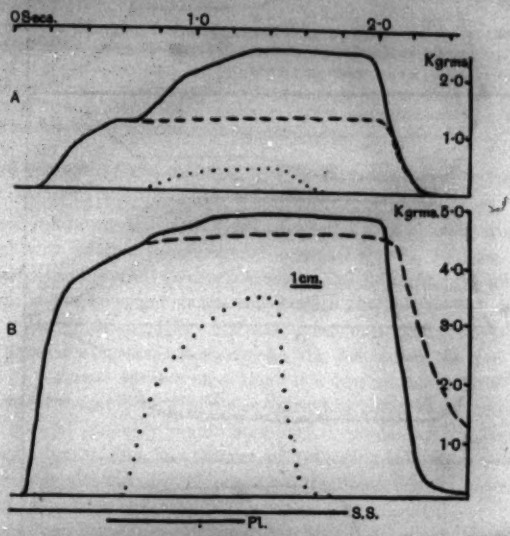

Fig. 8. *A*. Deafferented vastus medialis. Small sciatic nerve (*S.S.*) intercurrent in plantar nerve (*Pl.*). Both coils 26 cm. Control of small sciatic alone shown by dotted line and plantar by dashes. Signals shown below *B*.

B. As above but strength increased to 18 cm. coil for small sciatic and 16 cm. for plantar nerve. Magnif. = 550.

prolonged till the few non-occluded high threshold motoneurones attain threshold value.

It has been difficult to get definite evidence on the effect of concurrence on the rate of recruitment. A large number of reflexes were almost "d'emblée" in type and so for that reason little effect would be expected (Fig. 7 *B*), but even in these cases it has sometimes been possible to demonstrate an increase (*vide supra*, p. 100). With more gradual recruitment there has not seemed any marked effect of one reflex on the other. But in all our cases of gradual recruiting ascent there was evidence of inhibition as shown by rebound. This inhibition doubtless interfered with the summational process.

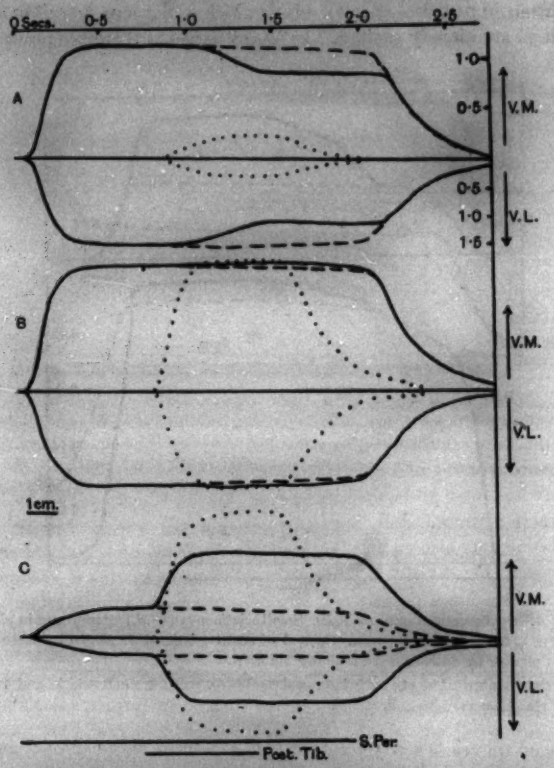

Fig. 9. Contractions produced simultaneously in deafferented vastus lateralis and vastus medialis by a crossed post-tibial nerve stimulation intercurrent in a crossed superficial peroneal nerve stimulation (*S. Per.*).

In *A*, coil for superficial peroneal nerve is on 14 cm. and for post-tibial nerve on 16 cm.

In *B*, coils for both nerves are on 14 cm.

In *C*, coil for superficial peroneal nerve is on 16 cm. and coil for post-tibial nerve is on 14 cm. In all controls of superficial peroneal nerve are shown by dashes and post-tibial nerve by dotted lines. The time and tension scales are identical for all. As shown by arrows at side, vastus medialis pulls upwards from a central zero line and vastus lateralis pulls downwards. Magnif. = 550.

When the intercurrent reflex is totally occluded, it is sometimes possible to see a definite prolongation of after-discharge of the background reflex.

Thus a peroneal reflex was completely occluded by a popliteal reflex, but the plateau after-discharge of the latter was then 182 and 190σ on two successive occasions, whereas a control of popliteal alone taken between these two observations possessed only 140σ plateau after-discharge. In other cases no such increase in after-discharge is present (Figs. 7 *A*, 9 *B*). In these cases, particularly in Fig. 9 *B* (see section on inhibition), inhibition is probably responsible for the absence of this lengthening.

(iii) *More complex effects exhibited by reflexes possessing both excitatory and inhibitory components.*

Crossed inhibition has often been associated with crossed excitation especially with internal saphenous or peroneal nerves. Increase of the strength of stimulus often greatly increases this inhibition. Even though inhibition be not apparent in the reflex when elicited singly it is often obvious in intercurrent (Fig. 9 *A*) or precurrent combinations with another crossed extensor reflex, *e.g.* in Fig. 5 *B*, when the precurrent stimulus to peroneal nerve ceases, there is a further rise in tension due to cessation of the inhibitory influence exerted by peroneal on some motor units. Rebound probably plays some part in producing this increased contraction, but it, too, is evidence of preceding inhibition ((25), (14)). Inhibition may also manifest its presence by reduction of an intercurrent test reflex (Fig. 9 *C*).

For the ipsilateral spinal reflex it has been conclusively shown that occlusion is a process distinct from inhibition though this may also be present (8). In the crossed extensor reflex inhibition has been a more common factor, so that the amount of true occlusion has often been difficult to estimate. Thus in Fig. 9 *B* the tension developed by post-tibial nerve alone is, if anything, slightly more than that produced by it when intercurrent in a superficial peroneal nerve reflex. We see from Fig. 9 *C* that a weak superficial peroneal definitely inhibits a post-tibial reflex of the same strength as above. It is obvious that in Fig. 9 *B* this inhibition must be present, but it is masked by the stronger excitation of superficial peroneal which almost exactly balances the inhibition, so that the tension developed by post-tibial when alone is practically equal to the total tension when it is intercurrent. We do not know whether this obliteration of inhibition under additional excitation takes place in those motor units which previously were inhibited or whether it is merely masked by the additional excitation of other motor units by superficial peroneal. Possibly both processes are partly concerned.

IV. Discussion.

We have seen that facilitation can occur between two stimuli which alone give no reflex response. Spread of stimulus can be excluded. Although no reflex response was produced, some afferent fibres in both nerves must have been excited. Moreover, for facilitation to be possible, some at least of the excited afferents must converge on the same neurone, be it the motoneurone of the extensor muscle in question or an internuncial neurone. The morphological basis of an extensive overlap has been demonstrated by Cajal(4) and others, and its physiological proof has been given(7) for the flexor reflex, and for the crossed extensor reflex by us. From our knowledge of the structure of the neurone it is evident that the actual locus of the facilitation must exist somewhere between the termination of the afferent fibre on the surface of the neurone "boutons terminaux" and the axon hillock ((29) p. 524). The subliminal (in the sense that it is insufficient to evoke a discharge down the axis cylinder of the neurone in question) excitation produced by a series of impulses arriving at the endings of one afferent must be able to reinforce the subliminal excitation produced from the other afferent, with the result that a discharge down the axis cylinder of the neurone in question is now produced. This argument is independent of any theory of excitation. Concurrent facilitation must therefore depend on the excitation of two afferent fibres both of them proper to each neurone facilitated. It is significant that it is especially easy to obtain facilitation between closely allied afferents, and that the overlap existing between them in the reflex centre is large, as shown by their powerful occlusion when stimulated strongly.

When one or both of the component stimulations independently produces a reflex response, the explanation of facilitation is exactly as above with, in addition, the possibility in the latter case of the existence of occlusion between some of the excited motor units. This would have the effect of making the facilitation appear less than it really was. Doubtless facilitation occurs in many cases where we have no evidence of its existence—being masked by simultaneous occlusion or inhibition.

The latent period of a reflex has been shortened when facilitation has occurred. It might be argued that this is due to the earlier response of the motor units added by facilitation, and that those which are excited by the stimulus in question independently, suffer no such abbreviation of latent period. Where (Fig. 5 *A* and *B*), despite but a small facilitation, there is a great shortening of latent period, it seems certain that many of the independently excited motor units are responding earlier. Latent

period especially as seen in the weak crossed extensor reflexes is more correctly spoken of as "summation period"(6). Its shortening by a concurrent stimulation of another afferent can, on the same reasoning as above, be explicable only by the excitatory impulses from this afferent being also incident on the same neurone. There the excitatory processes from each afferent must reinforce each other so as to cause an earlier discharge of impulses down the axon of this neurone.

With "sustained facilitation" it is also simplest to consider first those cases where neither stimulus alone causes any reflex response. Concurrent facilitation occurs, and it is found, when one stimulus is stopped, that there is no abrupt cessation or decrease of the contraction. In any particular neurone the reinforcing action of one subliminal excitation on the other does not cease when the arrival of impulses from its afferent has terminated. It is difficult to think that "delay paths" can be considered when the duration of the after-action is as long as 5 sec. The excitation of the neurone from one afferent is now able to maintain its supraliminal excitatory state owing to the effect of the previously excited other afferent. There has been a "surcharging" of the neurone during the concurrence. One is driven to the conception of Sherrington(29) that there is an accumulation of a substance *E*, be it ions or a chemical substance, at some locus in the neurone. During concurrence of stimulation this mounts up above threshold. If both stimuli stop simultaneously, then the excess is able to maintain excitation for some time (after-discharge). If, however, only one stimulus ceases, then the other is continually adding more *E*. This rate of addition of *E* is alone unable to reach threshold, but, when starting with a "surcharge" of *E*, it is able to considerably retard the falling of this below threshold. After-discharge is produced by supraliminal excitation of neurones persisting after the actual stimulus has ceased. The persisting excitation evident in sustained facilitation is not present as after-discharge when one of the facilitating reflexes is evoked alone. The after-discharge, in that case, soon ceases for the excitation of each neurone rapidly drops below threshold. Sustained facilitation must be the manifestation of that enduring subliminal excitation, which, by summing with the subliminal excitation of the same neurones by the background reflex, is able to maintain a supraliminal excitatory state in those neurones. Sustained facilitation is therefore, for the most part, an index of subliminal after-discharge. Its long duration is therefore easily intelligible.

A remarkable feature is that very often after one stimulus has ceased some considerable time elapses before any fall in tension occurs. *A priori*, with both stimuli acting concurrently all grades of supraliminal excitation

would be expected, some neurones being just above threshold. In these one would anticipate that the cessation of one stimulus would immediately result in the excitation falling below threshold. That this is very frequently not the case seems to indicate that the neurone itself has "inertia." It seems likely that, once the supraliminal excitatory state is set up, the neurone continues discharging down its axon even when its excitation has fallen below the initial threshold value. The frequency of plateau after-discharge in crossed extensor reflexes (deafferented) is of course also evidence in favour of this.

In an example already given under sustained facilitation it was shown how the "central drive" of a reflex, as measured by duration of plateau after-discharge, diminishes when one stimulus alone keeps up the reflex tension developed by two. One had therefore been sustaining excitation in all neurones at the expense of the surcharge accumulated during concurrence.

A constant test stimulus to one nerve evoked a much larger reflex response when preceded, at a constant interval, by a longer than when preceded by a shorter reflex evoked from another nerve. More excitatory charge had accumulated during the longer stimulation of the preceding nerve, and this had facilitated the after-coming test response. Moreover, the after-discharge of this was also augmented, showing that the central charge had been stored all this time.

It was shown that the facilitating power of a reflex first increased till the tension had about reached plateau height and then it gradually commenced to fall away. On the reasoning developed above this suggests that there was first an increase of E and then a gradual lowering of it in the subliminally excited neurones. Associated with this there is in time a reduction to below threshold of the E in those neurones supraliminally excited as is shown by the tension gradually falling. This process which we believe to be allied to fatigue needs much more investigation before its exact nature is ascertained. An alternative explanation would be that this fatigue process is a condition allied to adaptation, whereby the neurone becomes less responsive to the exciting influence of the E substance.

Our results have supported the distinction made (17), (18), (19) between the extensor type of reflex and the flexor type. Though we have frequently found crossed extensor reflexes approaching the "d'emblée" type, and have never seen the slow recruiting rise (in a deafferented preparation) unaccompanied by inhibition, a fundamental difference between the crossed extensor and the flexor reflexes lies in the former's marked power of summation and after-discharge as shown by concurrent and sustained

facilitation respectively. In this it is more closely allied to the fundamental properties displayed by the higher parts of the nervous system, than is the ipsilateral spinal flexor reflex which is simpler and which shows certain resemblances in behaviour to the transmission of impulses from motor nerve to muscle (17), (18). One is struck by the many similarities in behaviour between the scratch reflex (22) and the crossed extensor reflex. Thus temporal and spatial summation and after-discharge are in both striking features. One has yet to discover whether their difference from the flexor type of reflex is due merely to their more complex reflex path, or if there is some special summational property of either the internuncial or motor neurone.

V. Summary.

1. In a preliminary account of crossed extensor reflexes in the de-afferented preparation the various modifications of latent period, recruitment, and after-discharge are described. Recruitment is often found to be a very rapid process.

2. Crossed extensor reflexes of various fractions of quadriceps are recorded simultaneously, and it is found that, while vastus lateralis and vastus medialis are similar in behaviour, rectus femoris presents many striking differences when compared with a simultaneously recorded vastus medialis. The peculiar behaviour of rectus is possibly associated with its specialised function as a double joint muscle.

3. In contrast with flexor reflexes facilitation is a very prominent feature in the interaction of crossed extensor reflexes.

Two sub-groups are described:

(*a*) When the effect is exerted by one reflex on another concurrently excited (concurrent facilitation).

(*b*) When it appears as the after-effect of one reflex on another (sustained facilitation).

4. The facilitating power of one weak reflex on another is not maximal until its recruitment is almost complete, and soon after this it again diminishes, apparently owing to some fatigue process. There is, therefore, an optimal point of facilitation.

5. An invariable shortening of latent period accompanies facilitation.

6. In many respects occlusion, as exhibited in the interaction of two crossed extensor reflexes, is found to have features in common with a similar phenomenon in flexor reflexes.

7. The presence of inhibition in some reflexes and the possible influence of its presence on interaction is considered.

8. The nature of facilitation is discussed and, from the data obtained, one is compelled to postulate the accumulation of some excitatory substance in the neurone. The Sherrington conception is thus supported.

9. Sustained facilitation is shown to be an index of subliminal after-discharge.

10. The evidence obtained also supports the distinction which Liddell and Sherrington have made between the extensor and flexor types of reflex.

We wish to express our thanks to Sir Charles Sherrington and to Dr Denny-Brown for valuable advice, and to the Christopher Welch Trustees for defraying the cost of the photographic material.

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THE DISCHARGE OF IMPULSES IN MOTOR NERVE FIBRES. Part II. The frequency of discharge in reflex and voluntary contractions.

By E. D. ADRIAN and D. W. BRONK (*Fellow in Medicine, National Research Council, U.S.A.*).

(*From the Physiological Laboratory, Cambridge.*)

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Erratum

P. 143, Fig. 20. *For* 'peroneal' *read* 'popliteal'.

chest during normal breathing (in the rabbit anæsthetized with urethane) the frequency of the impulses in each nerve fibre was very low (20–30 a sec.)—so low in fact that the muscle fibre response would be an incompletely fused tetanus. When the force of inspiration was increased the frequency of discharge rose to 50–80 a sec. Since there was no clear evidence that more neurones came into play with the stronger contraction, we concluded that the contraction of the diaphragm is regulated mainly by varying the frequency of the motor impulses over a range which would give a varying degree of fusion and summation of the contractile waves in the muscle fibres.

leading from entire nerve trunk, *A*, and single nerve fibre, *B*.

We have now applied the same method to determine the frequency of the motor discharges in various reflexes of the hind limb in the cat. We have found the same very low frequencies with feeble contractions and the same increase when the contraction becomes more forcible,

8. The nature of facilitation is discussed and, from the data obtained, one is compelled to postulate the accumulation of some excitatory substance in the neurone. The Sherrington conception is thus supported.

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THE DISCHARGE OF IMPULSES IN MOTOR NERVE FIBRES. Part II. The frequency of discharge in reflex and voluntary contractions.

By E. D. ADRIAN and D. W. BRONK (*Fellow in Medicine, National Research Council, U.S.A.*).

(*From the Physiological Laboratory, Cambridge.*)

In Part I of the present series(1) we described a method of recording the normal discharge of motor impulses in single fibres of the phrenic nerve. The method consists in cutting through all but one of the active motor fibres of the nerve and recording the action currents from this one fibre by electrodes placed on the nerve trunk below the divided area (cf. Fig. 1 *B*). As a rule at least three or four fibres were left uncut, but in five experiments the impulses recorded during the respiratory discharge formed a single, fairly regular series and were presumably derived from a single source. We found that during normal breathing (in the rabbit anæsthetized with urethane) the frequency of the impulses in each nerve fibre was very low (20–30 a sec.)—so low in fact that the muscle fibre response would be an incompletely fused tetanus. When the force of inspiration was increased the frequency of discharge rose to 50–80 a sec. Since there was no clear evidence that more neurones came into play with the stronger contraction, we concluded that the contraction of the diaphragm is regulated mainly by varying the frequency of the motor impulses over a range which would give a varying degree of fusion and summation of the contractile waves in the muscle fibres.

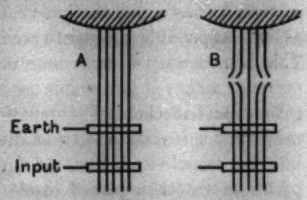


Fig. 1. Arrangement of electrodes for leading from entire nerve trunk, *A*, and single nerve fibre, *B*.

We have now applied the same method to determine the frequency of the motor discharges in various reflexes of the hind limb in the cat. We have found the same very low frequencies with feeble contractions and the same increase when the contraction becomes more forcible,

though with certain reflexes there is also a marked increase in the number of fibres in action. The results of this work are given in Section I; in Section II we describe confirmatory experiments in which the action currents of single groups of muscle fibres are recorded during reflex contractions in the cat and voluntary contractions in man.

Section I. Impulses in single motor nerve fibres in reflex contractions of the hind limb.

Method.

All the experiments in this section were made on cats which were deeply anæsthetized and then either decapitated by Sherrington's technique or decerebrated by the trephine method with or without previous spinal transection. One hind limb was fixed by a drill through the lower end of the femur and by clamps in the region of the pelvis and foot. In some experiments the other limb was immobilised by nerve section, but as a rule the innervation of both limbs was interfered with as little as possible in order to secure the greatest possible reflex activity. This is necessary because our method of recording the nerve action currents makes it impossible to use electrical stimulation to elicit the reflexes and mechanical stimulation is usually much less effective. The nerve under investigation was divided as close as possible to its entry into the muscle and dissected for 2–3 cm. proximally. The whole preparation was then placed inside a metal screening box connected to earth and the nerve (irrigated repeatedly with warm Ringer) was slung on two paint brush electrodes, the proximal connected to earth and the distal to the input of the three valve amplifier (Fig. 1 *A*). To avoid electrical artefacts the reflexes were produced by pinching the feet or moving the limbs or head with a pair of forceps made of vulcanite with long handles which extended outside the screening box. Permanent records of the reflex discharge in the nerve were made with the capillary electrometer, but in making these we have been guided more and more by the information obtained by amplifying the action currents still further and leading them to a loud speaker (cf. Part I, Fig. 2). By listening to the nerve discharge throughout the experiment we have been able to save a great deal of time and photographic material and to detect various points which would almost certainly have been missed had we relied entirely on photographic records.

After a few preliminary observations on the reflex discharge from the entire nerve trunk, the preparation was removed from the screening

box and the nerve laid across a small glass platform and focussed with a binocular dissecting microscope. The progressive division of the nerve fibres was carried out by the method described in Part I. The final stages of the operation were controlled by repeated observations of the action currents in the nerve distal to the section and eventually, if all had gone well, the stage was reached at which the action currents form a single series. As in the experiments on the phrenic this stage was usually reached when there were still three or four undivided fibres bridging the gap in the nerve, but it is clear that the action currents are due to only one of these fibres, since the impulses form a single series and further division either suppresses the discharge entirely or else leaves it unchanged. When the division of the nerve had been successfully carried out, the preparation was placed once more in the screening box and the reflex discharges were photographed with the electrometer. The intact connective tissue sheath of the nerve preserves the uncut fibres from further damage during manipulation and the character of the discharge remains unchanged for periods of 2–3 hours. There is, of course, a considerable risk of carrying the section of the nerve too far and missing the single fibre stage, but only about one in three of our experiments have failed from this cause.

It will be realised that for this measure of success the nerve must be carefully chosen. The most suitable size appears to be one containing about 150 fibres at the point where the section is to be made, and for ease in manipulation it must be possible to dissect out a length of 2·5 to 3 cm. of the nerve. We have found various small nerves which comply more or less with these conditions. They are the nerve to the peroneus longus, the upper or lower branch to the tibialis anticus, a branch of the anterior crural which runs down on the mesial surface of the vastus lateralis and supplies the lower third of that muscle and another branch of the anterior crural supplying the vastus medialis. Those to the peroneus longus and tibialis anticus have been used for the study of the flexion reflex, and those to the thigh muscles for the extensor reflexes. The former have been studied exclusively in the spinal and the latter in the decerebrate preparation.

Results. (1) *The flexion reflex.*

When the foot of a spinal animal is pinched, the tibialis anticus contracts strongly and flexes the ankle joint: the peroneus longus also contracts but not so strongly, its function being to assist the flexion and to prevent inversion of the foot (cf. Sherrington(2)). Both muscles

are supplied from the main peroneal nerve trunk, the arrangement of the terminal branches in the cat being shown in Langley's diagram (3).

Nerve to peroneus longus. Our first experiments were made on the nerve to the peroneus longus. This can be secured with a minimum of dissection and freed from the main peroneal trunk for a considerable length (3–4 cm.). A section of the nerve stained in osmic acid contained 129 fibres of 10μ or more in diameter and 55 under 10μ. This represents the whole nerve supply to the muscle, and as the nerve must be cut distally to allow of the electric recording, no afferent impulses can pass from the muscle to the spinal cord. The other nerves we have used do not form the entire supply of the muscles they innervate, but here too it must be remembered that some of the afferent supply from the muscle had to be sacrificed. Fortunately the evidence brought forward in Section II shows that the main result is quite unaffected by this factor.

The nerve to the peroneus longus was successfully brought to the single fibre stage in three experiments. A record of the beginning and middle of a reflex discharge is shown in Fig. 2. The stimulus was a

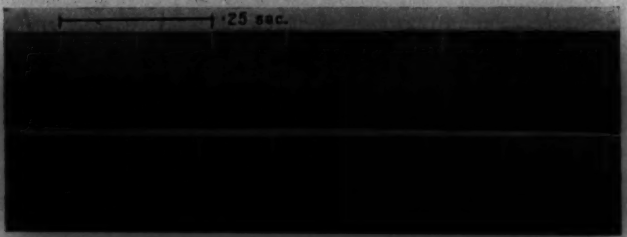


Fig. 2. *Exp.* 2. Decapitate cat. Nerve to peroneus longus, final stage of dissection. Flexion reflex produced by pinching foot. Above, beginning of discharge; below, 1 sec. later. Time marker (white lines at the top of the record) gives 0·125 sec. intervals.

gradually increasing pressure on the foot and it will be seen, (*a*) that the frequency of the impulses rises slowly at the beginning of the contraction, (*b*) that at the height of the discharge the frequency is only 16 a sec. and (*c*) that although the impulses evidently form a single series the regularity of the discharge is by no means absolute—in fact the intervals between successive impulses may vary by as much as 30 p.c. from the average value. The record in Fig. 2 is typical of a large number made in the course of the three experiments; in all of them the frequency of discharge remained below 30 a sec. even with the strongest stimulation,

and in all the frequency rose and declined gradually. The evolution of the discharge may be seen in Fig. 3 which gives two curves showing

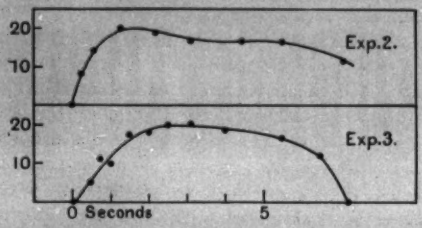


Fig. 3. Curves showing frequency of impulses per sec. in nerve fibre to peroneus longus during flexion reflex. *Exp.* 2. Decapitate preparation. *Exp.* 3. Decerebrate with spinal transection.

the frequency at different times after the beginning of the contraction. As the muscle has lost its nerve supply we cannot compare these curves with those of the tension developed in the contraction, but if all the nerve fibres behave alike the contraction would rise and decline gradually and would never be very powerful. As three nerve fibres selected at random in three different animals were all found to give a low frequency discharge of the same kind it may safely be assumed that all the motor fibres behave alike, and observations of the contraction before the nerves were cut showed that the stimulation we employed did produce a feeble reflex contraction of gradual onset and decline.

The contractions appeared to develop smoothly, but it is clear that in the group of muscle fibres innervated by one nerve fibre the contraction must have been an incomplete tetanus, in fact at the beginning of the response it would be little more than a succession of twitches. The average tension in the whole muscle would rise gradually as the frequency of the discharge increases, at first from the increased frequency of these isolated twitches and later from the summation of the contractile effect in the individual muscle fibres.

Nerve to tibialis anticus. Three successful experiments of the same type were made on one or other of the two main nerve twigs to the tibialis anticus. Fig. 4 shows the reflex discharge in the nerve in one of these at different stages of the experiment. In the top record the nerve is intact (though cut distally at its junction with the muscle) and the electrometer is shunted to record the very large fluctuations of potential. The middle record was obtained after about nine-tenths of the fibres

had been cut through, and the lowest, which shows a single series of action currents, was made after division of all but three fibres. One of

Fig. 4. *Exp.* 6. Decapitate cat. Nerve branch to tibialis anticus. Records made at height of flexion reflex. *A*, whole nerve; *B*, partially divided; *C*, final stage, three fibres remaining. Single impulse series at 30 a sec.

these was only 5μ diam., the other two were over 10μ, and one of them was bent irregularly and presumably took no part in the discharge. The three records are not strictly comparable, but they were all obtained at the height of the discharge with a strong stimulus to the foot. It will be seen that the frequency of the impulses in the single fibre is distinctly higher than in the nerve to the peroneus longus, and this is shown more clearly in Fig. 5 which gives the frequency at each moment throughout a reflex contraction lasting 9 secs. The highest frequency reached in this discharge is 40 a sec., not high enough to produce a completely fused tetanus in the muscle fibres, but twice as high as in the nerve to the peroneus longus. In another experiment the maximum rate was 44 a sec.

The higher values reached with the nerve to the tibialis are evidently related to the fact that we are dealing with a much stronger contraction, the tibialis being a prime mover in the flexion of the ankle and the peroneus longus an accessory. But with weak stimulation the frequency may be just as low as in the nerve to the peroneus longus, and it is

possible to vary the frequency of the reflex discharge at will by altering the pressure of the forceps on the foot. After a strong stimulus the

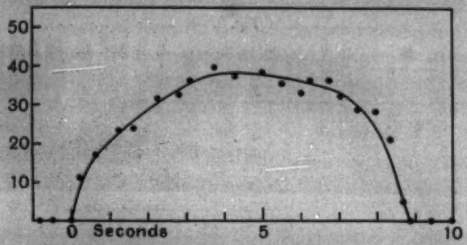


Fig. 5. *Exp.* 6. Curve showing frequency of impulses per sec. in nerve fibre to tibialis anticus during flexion reflex. Decapitate preparation.

after discharge may last for several seconds at a frequency which gradually declines to 10 a sec. or less. In the intervals between stimulation the nerve fibre is usually quiet. On one occasion however we found a persistent discharge at about 15 a sec. (estimated very roughly by ear) and were finally able to trace it to the fact that the foot had become cold. Warming the foot by pads wrung out in hot water abolished the discharge and it returned again whenever the pads were removed and the foot allowed to cool.

(2) *Extension reflexes, nerves to quadriceps.*

The nerve fibres of the anterior crural supplying the quadriceps were studied in three successful experiments on decerebrate preparations. Two of these gave a strong extension on mechanical stimulation of the contralateral foot; in the third we failed to elicit the crossed extension reflex, but we were able instead to produce a strong extension of both legs by moving the head from the horizontal position to that in which the long axis makes an angle of 45° with the horizontal, the preparation lying on its back. This is the position in which the tonic effect of the labyrinths is at its maximum, and the extension occurring in this experiment is therefore an example of the tonic labyrinthine reflex.

The main features of the discharge in single fibres of the anterior crural nerve are, first, that it may persist at a frequency of 10–25 a sec. for long periods in the absence of external stimulation, and, second, that during a movement of extension the discharge rises fairly rapidly to frequencies as high as 90 a sec.

A record of the persistent discharge is given in Fig. 6 from the pre-

paration showing active labyrinthine reflexes. The nerve was the lowest branch to the vastus lateralis[1], all the other nerves of the leg being

Fig. 6.

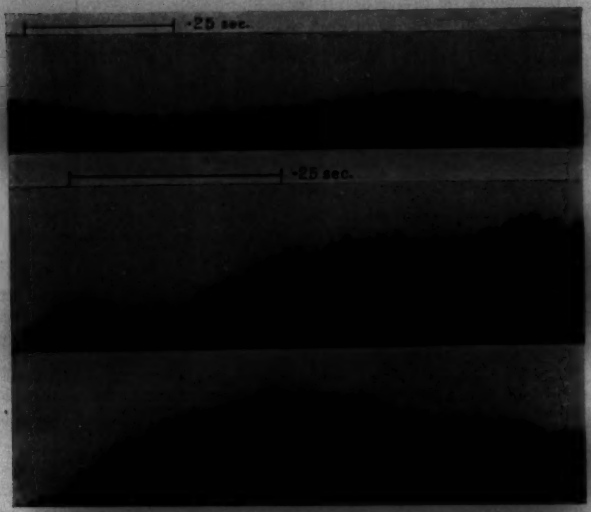


Fig. 7.

Fig. 6. *Exp.* 11. Decerebrate cat. Nerve to vastus lateralis. Several fibres intact. Portion of long record showing persistent "tonic" discharge in one fibre at 25 a sec. Increase of contraction due to stimulating labyrinths brings more fibres into play.

Fig. 7. *Exp.* 10. Decerebrate cat. Nerve to vastus medialis. Final stage. Crossed extension reflex produced by pinching contralateral foot. *A*, beginning of contraction; *B*, height of discharge. Frequency, 65 a sec.

intact. The discharge could be inhibited by pinching the ipsilateral foot but returned again as the extensor tone reasserted itself. Similar discharges were found in the other two experiments, but in these the extensor tone was less and the discharge was sometimes absent for long periods, to return again after manipulation of the leg.

We have no doubt that these persistent low frequency discharges are the cause of the "tonic" contraction of decerebrate rigidity, and

[1] A section of this nerve gave 103 large and 49 small fibres in the region selected for dissection

that the "tone" of the quadriceps is in fact maintained by incompletely fused tetanic contractions in various groups of muscle fibres. But it might be objected that the nerve discharge as it appears in our records may have been changed from its normal character owing to the section of the sensory fibres. This objection applies, of course, to all our records from divided nerves, but it becomes of especial importance in the case of reflexes which may be maintained by the afferent impulses from the muscle. The difficulty can be overcome by experiments of a different kind, but these and the general discussion of "tonic" innervation must be reserved for Section II.

When movements of extension are produced by stimulation of the contralateral foot the frequency of the impulses rises fairly rapidly to values which range from 60 to 90 a sec. Fig. 7 gives two portions of a record from a nerve twig to the vastus medialis, and Fig. 8 shows one

Fig. 8. ABOVE. *Exp.* 10. Decerebrate cat. Nerve to vastus medialis. Final stage of dissection. Record at height of crossed extension reflex. Frequency, average 63 a sec. Shortest interval between impulses, 82 a sec.

BELOW. *Exp.* 6. Decapitate cat. Nerve to tibialis anticus. Final stage. Record at height of flexion reflex. Frequency, 31 a sec.

Time marker gives ·01 sec. intervals.

made on a rapidly moving plate at the height of a discharge with maximal stimulation. Here the average rate is 82 a sec., and the shortest interval between successive responses gives a rate of 95 a sec.—the most rapid we have found in any experiment. This record is one of many which we have made from time to time to satisfy ourselves that the electrometer excursions are due to single impulses and not to groups

occurring in rapid succession. Inspection of the record shows no evidence of this and the analysis gives a succession of simple monophasic action currents. The lower record is from the nerve to the tibialis anticus during the flexion reflex.

Fig. 9 gives the frequency curves for two discharges in the crossed

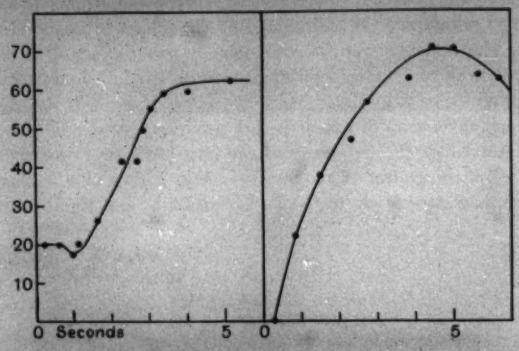


Fig. 9. *Exp.* 10. Frequency of impulses per sec. in nerve fibre to vastus medialis during crossed extension reflex.

extension reflex, one starting from the background of a persistent low frequency discharge and one from a quiet nerve. A comparison with Figs. 3 and 5 shows the much greater frequencies which are reached in these extensor reflexes.

We have not examined enough reflexes to say whether the difference in frequency is characteristic of flexor and extensor reflexes or whether it is merely due to the fact that the contractions we were able to elicit were more nearly maximal in decerebrate crossed extension than in spinal flexion. There is however another point of difference which may have more significance, and this concerns the grading of contraction in the various reflexes.

Grading of contraction. In the individual nerve fibres of the phrenic we found that the frequency of the impulses varied over a range from about 15 to 90 a sec. Electrical stimulation of the whole nerve at frequencies ranging from 15 a sec. upwards gave a series of contractions of gradually increasing force. With low frequencies the contraction was an incomplete tetanus and the increase in force with higher rates was due to the more and more complete summation of the individual twitches.

The contraction became maximal or nearly so when the rate was 50–60 a sec. Thus the frequency of the reflex discharge varied over just the range required to produce graded contractions in the diaphragm.

In the present experiments we find the reflex frequencies ranging from as low as 5 to as high as 90 a sec. The effect of stimulating the

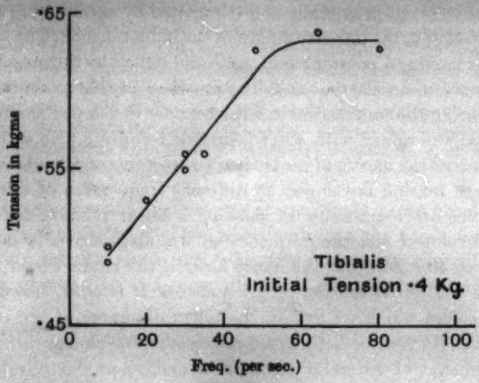


Fig. 10 *A*.

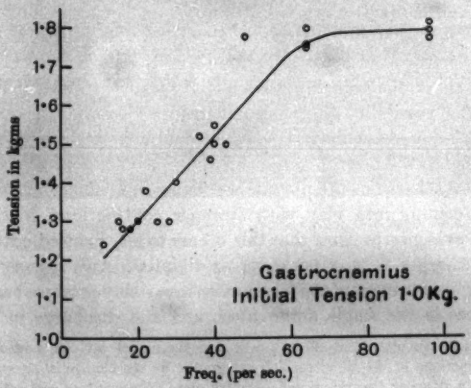


Fig. 10 *B*.

Fig. 10. Tension developed in leg muscles with stimulation of motor nerve at various frequencies. Decerebrate. *A*, tibialis anticus; *B*, gastrocnemius and soleus. Contractions not strictly isometric.

nerve to the tibialis anticus with rhythmic induction shocks at different frequencies is shown in Fig. 10 *A*. The quadriceps gave too powerful a contraction for the very simple myograph arrangement which we used, but we have records made by stimulating the popliteal nerve and measuring the pull of the combined gastrocnemius and soleus (Fig. 10 *B*). The nerves were cut proximally and stimulated by shocks from a rotating contact breaker in the primary circuit of the induction coil. The myograph was a lever moving a pointer over a scale and pulled by the muscle against the resistance of an elastic band. The muscle was able to shorten 5 mm. or more during the contraction and the tensions developed are lower than those usually obtained with strict isometric recording. No attempt was made to reduce the inertia of the lever since we were concerned merely with the average tension developed at different frequencies of stimulation. Both curves are remarkable in showing a linear relation between the tension developed and the frequency of stimulation over a range from about 15 to 40 a sec., but the exact form of the curve would no doubt vary with the initial tension on the muscle. It is clear, however, that the frequencies which we find in the reflex discharge of the individual nerve fibres lie mainly within the range where a change in frequency alters the level of muscular summation and so produces a change in the force of contraction. With more perfect myographic recording Liddell and Sherrington(4) have found small fluctuations in tension corresponding to stimulus rhythms as high as 90 a sec., so that even the highest frequencies we have found in the individual nerve fibres must fall short of producing a completely smooth tetanus. We cannot exclude the possibility that higher rates of discharge may sometimes occur, but an increase above 100 a sec. could have little or no functional value as far as the muscle is concerned.

In the discharge of the phrenic we found no evidence of more motor neurones coming into play with stronger contractions, and we have found no evidence to show that this occurs to any marked extent in the reflex discharges to the peroneus or tibialis[1]. Any sensory stimulus producing a movement of the leg muscles invariably produced a discharge of impulses in the single nerve fibre, and the discharge in this fibre

[1] Electrical stimulation of afferent nerves in the spinal cat can evidently produce reflex contractions in the tibialis which are graded by the change in number of active fibres. But a stronger electric stimulus excites more afferent fibres without altering the frequency of the impulses in each fibre, whereas pinching the foot more strongly will probably increase the frequency of discharge from the receptors without much increase in the number in action. This may account for our finding only that type of muscular grading which depends on increased frequency of discharge.

appeared to last throughout the period of contraction. To investigate the time relations more closely we made one experiment in which the electric responses of the entire nerve trunk above the damaged region were compared with those of the single nerve fibre below it.

The former were magnified by a separate three valve amplifier followed by a power amplifier which led to a loud speaker movement of the "balanced armature" type. The vibrating reed carried a small glass pointer which projected into the eyepiece of the capillary electrometer. The excursions of the pointer, magnified 40 times on the film, were rarely greater than 1 mm. and considerable distortion was introduced by the amplifying and recording system, but a comparison with simultaneous electrometer records from the same leads showed that the system was adequate to signal the beginning and end of the discharge in the entire nerve. The loud speaker movement forms a convenient signal for electrical stimulation (see p. 141).

In this experiment the discharge in the single nerve fibre coincided in time with the discharge of the whole nerve, *i.e.* the single fibre, chosen at random, did not come into play any later than the other fibres. Evidence pointing in the same direction was obtained from records

Fig. 11. *A. Exp.* 7. Decapitate cat. Nerve to tibialis anticus, several fibres intact. Beginning of flexion reflex.

B. Exp. 13. Decerebrate cat. Nerve to vastus lateralis, several fibres intact. Beginning of crossed extension reflex. Many fresh fibres come into play ·3 sec. after the first.

made from nerves in the earlier stages of the dissection when several fibres were still active, for these showed little sign of greater complexity in the discharge (*i.e.* more fibres in action) in the middle than at the beginning or end of the discharge, whatever the stimulus. An example of one such record is given in Fig. 11 *A*. The records obtained from the muscle itself and discussed in Section II show that one group of muscle

fibres may occasionally come into play before the majority, but they support the view that in these reflexes there is very little to change in the number of active neurones after the contraction has begun.

In the case of the quadriceps, however, there is no doubt that the number of fibres in action increases progressively as the contraction develops. This can be seen at once from Fig. 11 *B* which gives a record from the nerve to the vastus lateralis before the final stage of the dissection was reached. A single series of impulses appears at the beginning of the contraction, but at a later stage many more neurones come into play and the action current record becomes increasingly complex. Subsequent dissection gave the usual single fibre discharge without the additional impulses. The fact that some neurones only come into play with strong contractions may explain why we do not always find the persistent "tonic" discharge when we have reached the single fibre stage. All our preparations showed occasional low frequency discharges in the absence of external stimulation, but we have not enough evidence to say whether the same nerve fibre is used at one time for maintaining the tone of the extensors and at another time becomes active only at the height of a powerful contraction. The significance of these results will be discussed later in Section II.

Section II. The electric response in small groups of muscle fibres.

The experiments on the divided nerve are technically difficult and in interpreting their results we have to reckon with the fact that some or all of the sensory impulses from the muscle are prevented from reaching the cord. But it is unlikely that they will produce a radical change in the frequency of the discharge, and this is so low that the muscle fibres should certainly respond to every impulse reaching them from the nerve. Consequently the electric responses in the individual muscle fibres should give just as accurate a measure of the nerve fibre frequency as the record made from the nerve itself. We have not attempted to dissect out individual muscle fibres, or groups supplied by a single nerve fibre, since most of the information we need can be readily obtained by the use of very small electrodes buried in the substance of the intact muscle. The principle involved can be seen in the diagrams in Fig. 12.

If two point electrodes a and a' are placed in a muscle at a distance apart which is large compared with the diameter of the muscle fibres (as in Fig. 12) they will record the algebraic sum of the potential changes in many fibres besides those in contact with the electrodes, for a potential

gradient in a fibre some distance away, as at x, will produce a considerable potential difference between the a and a'. But if the electrodes are

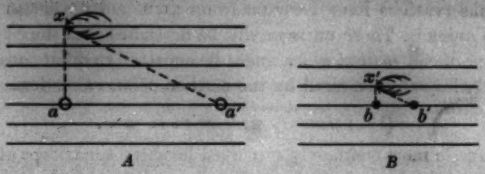


Fig. 12. To show greater localisation due to electrodes of small dimensions. (See text.)

brought very close together (b, b', Fig. 12 *B*) their sphere of influence will be reduced proportionately and the potential gradient must now occur at x' if it is to have the same effect as that at x when the electrodes are at a and a'. After trials of various types we finally adopted the arrangement shown in Fig. 13. An enamelled copper wire, No. 36 gauge,

Fig. 13. Concentric needle electrode.

i.e. 193μ diam., is passed up the centre of a small hypodermic steel needle and held in position by a plug. The exposed end of the copper wire projects into the orifice of the needle and forms one electrode. The needle itself forms the other. The gap separating the two electrodes is very small, little more than the thickness of the enamelled covering of the wire, but the wire has a diameter 3–4 times as large as that of a muscle fibre, and if the end is ground off obliquely to fit the end of the needle the exposed area is larger still. Sharper localization is secured by using two smaller wires (No. 44 gauge, *i.e.* 81μ diam.) placed side by side in a hypodermic needle, but this arrangement has certain practical disadvantages which are not present with the concentric type. In the latter the body of the needle is connected to earth and the wire to the grid of the amplifier, and as the needle has a relatively large surface it provides a low resistance path from the animal to the earth. This makes it possible to use electrical stimulation and to work without the careful electrical shielding which is necessary when both leads are of high resistance, but the localization is not sharp enough to distinguish the responses of individual fibres, or fibre groups, in powerful contractions where every fibre is in action, and our records with the concentric type of electrode give most information in relatively feeble contractions

of the type in which the motor neurones do not all come into play at once.

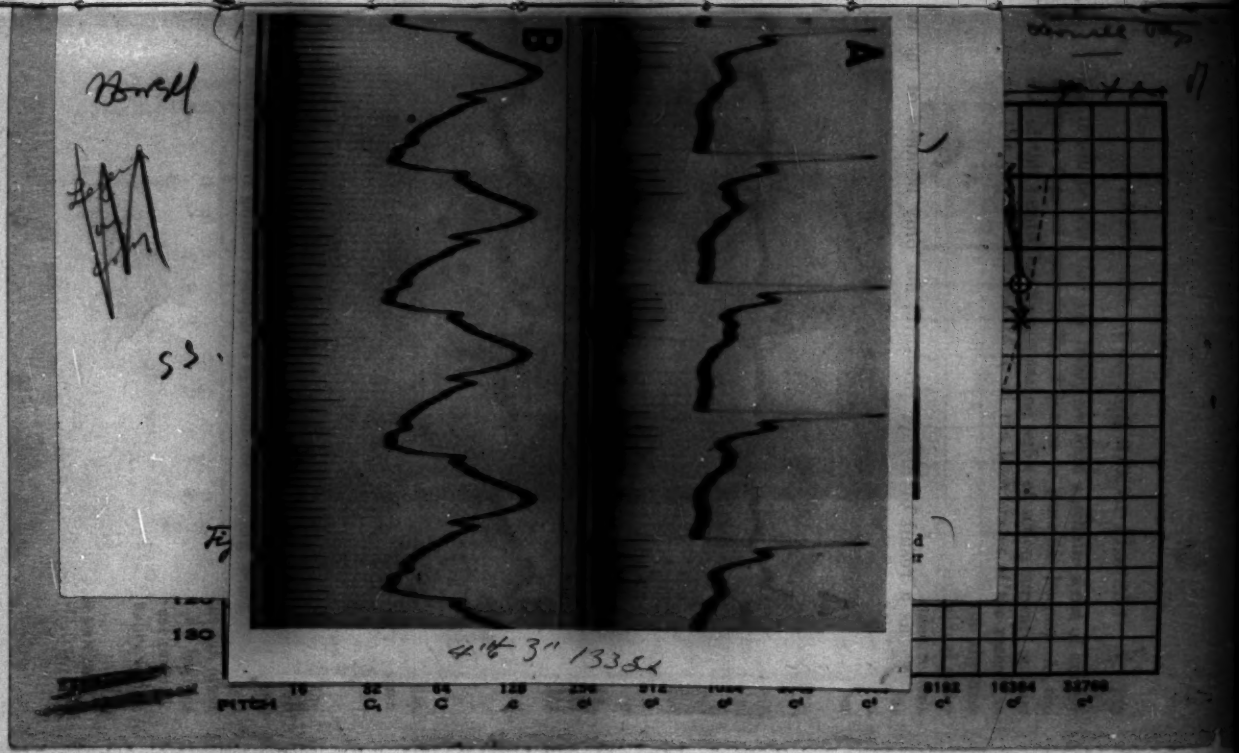
Our observations have been made on man and on spinal and decerebrate animals. Those on man will be described first since they deal with a type of contraction which might be expected to differ considerably from the reflexes investigated by the method of nerve section.

(1) *Voluntary contraction in man.*

The muscle most commonly employed has been the triceps of the left arm. The needle electrode is thrust into the muscle with the central wire connected to the input of the amplifier. As a rule the electrometer is shunted to one-sixth or less of its normal sensitivity, for the action

Fig. 14. Action currents in human triceps (E. D. A.) recorded with concentric needle electrodes during gradually increasing voluntary contraction. *A*, beginning of contraction; *B*, follows on *A*; *C*, powerful contraction.

currents are much larger than those of nerve. When the muscle is completely relaxed the record shows no electric changes. With a slight degree of voluntary contraction sharply isolated action currents appear at a rate which may begin as low as 6 a sec. and rises gradually as the contraction develops. Records of these low frequency discharges are given in Fig. 14. In Fig. 14 *A* the short time relations and the simple contour of each response show that it must be due to a single muscle fibre or to a group of muscle fibres acting in unison and so presumably innervated by a single nerve fibre. In agreement with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of the type in which the motor neurones do not all come into play at once.

Our observations have been made on man and on spinal and decerebrate animals. Those on man will be described first since they deal with a type of contraction which might be expected to differ considerably from the reflexes investigated by the method of nerve section.

as a result of higher pressure, not only because of the in-

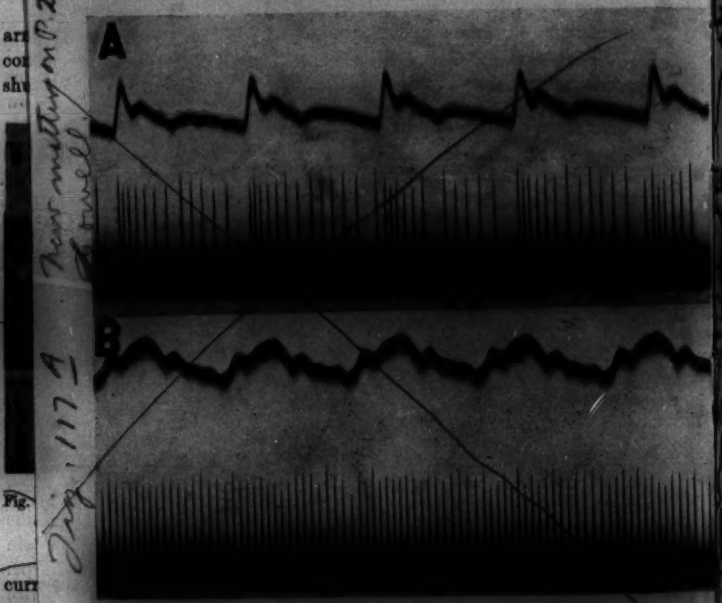
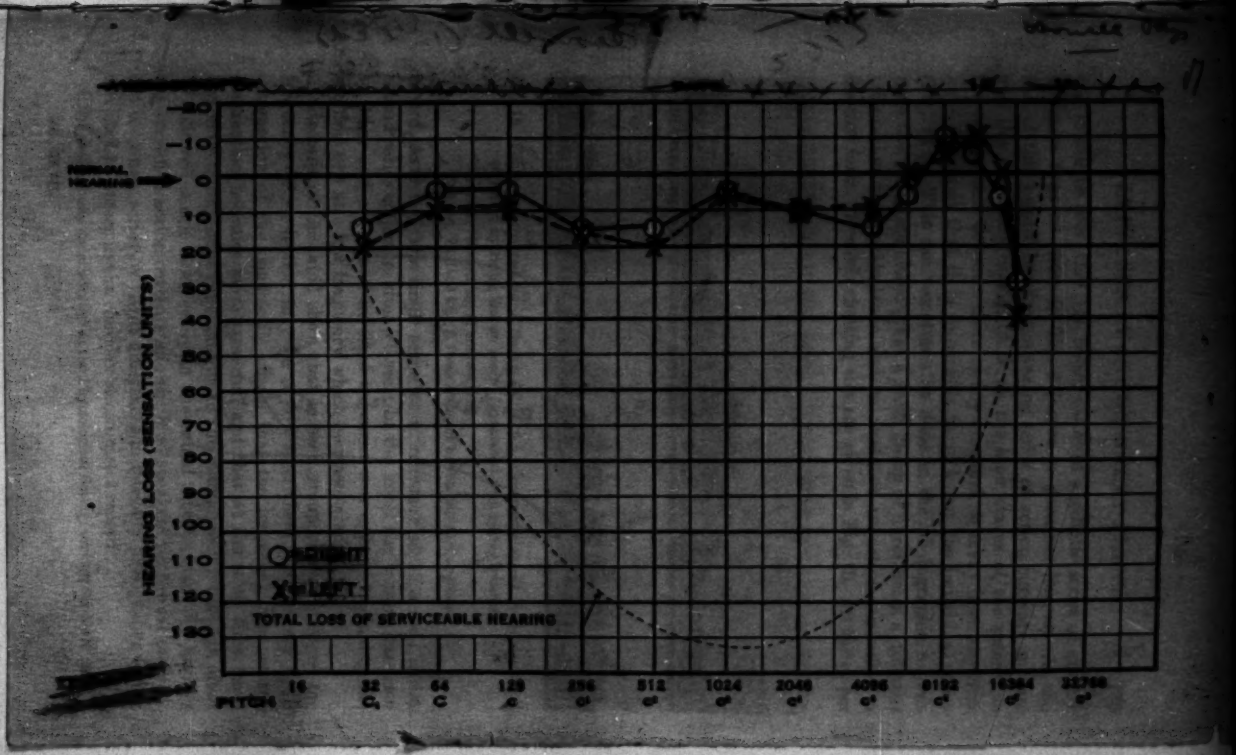


Fig. 6 The increased discharge of impulses resulting from variations in mea

at a rate which may begin as low as 6 a sec. and rises gradually as the contraction develops. Records of these low frequency discharges are given in Fig. 14. In Fig. 14 *A* the short time relations and the simple contour of each response show that it must be due to a single muscle fibre or to a group of muscle fibres acting in unison and so presumably innervated by a single nerve fibre. In agreement with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HEARING LOSS (SENSATION UNITS)
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TOTAL LOSS OF SERVICEABLE HEARING
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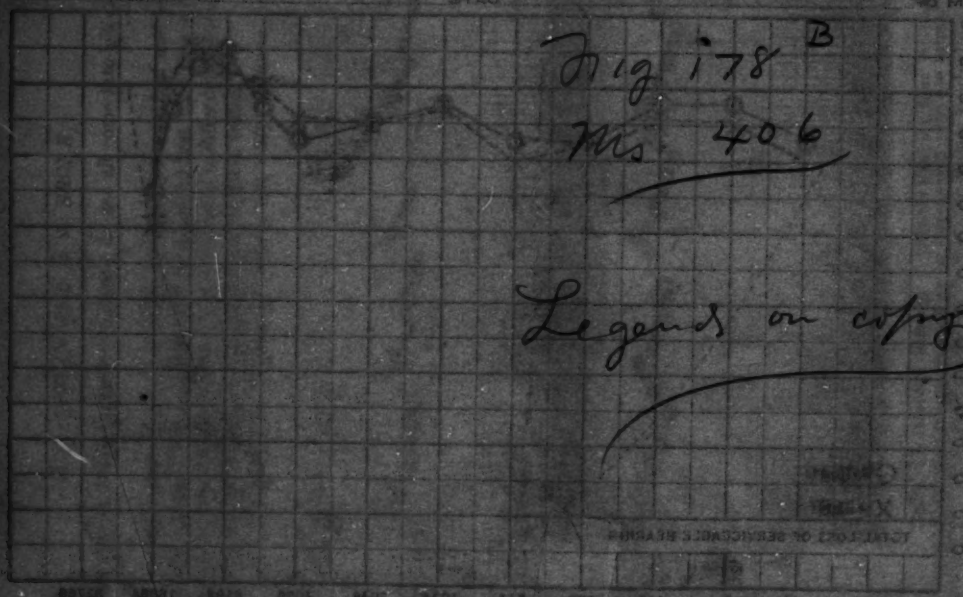

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this it will be seen that the responses form a definite series of exactly the same type as those in Fig. 2 (nerve to peroneus longus). At the end of this record, however, a second series of action currents appears and this continues in Fig. 14 *B*: in the later stages of the record, as the contraction becomes more nearly maximal, so many fresh rhythms have appeared that it is impossible to pick out the individual series (Fig. 14 *C*). This can sometimes be done in the photographic records from the fortunate chance that a fresh group of fibres bears a different orientation to the electrode and so produces an action current of a distinct form. Records showing this are given in Fig. 15. In the upper

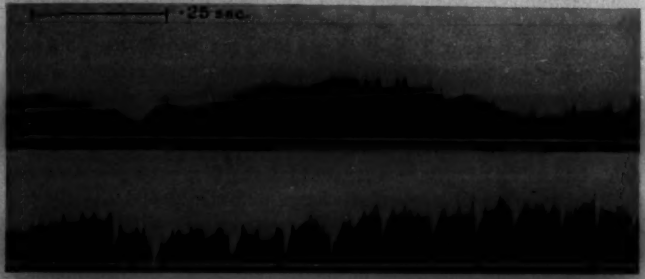

Fig. 15. Voluntary contraction of triceps (E. D. A.). Concentric needle electrodes. Pull recorded by rising line on record (connected with spring balance).

ABOVE. Beginning of contraction.

BELOW. Pull = 2 kg. A fresh series of action currents appears.

record there is first a single series at a frequency of 14 a sec., and a second series comes in after 0·6 sec. In the lower the tension has risen to 2 kg. and a series of inverted responses now appears beginning like the first two at a low frequency which increases gradually.

Records of this kind show that in each group of muscle fibres the frequency of excitation may be so low that there can be very little summation of the contractile effect; that the frequency rises as the contraction becomes more powerful, and that at the same time more and more fibre groups come into play. The individual rhythms can often be followed much more easily in the sound records made by leading the amplified action currents into the loud speaker[1], for the ear can pick out each new series by slight differences in intensity and quality which are hard to detect in the complex electrometer record.

[1] The method was demonstrated to the Physiological Society on October 13th, 1928.

We have been at some pains to satisfy ourselves that no fresh type of electric response enters into our records at any stage in the contraction and that the apparent complexity with strong contractions is merely due to the activity of more and more fibre groups. In view of the results obtained from the single nerve fibres this could scarcely be doubted. But a different suggestion has been put forward by Wachholder(5) and the results on which his view is based make an interesting commentary on the analysis of the electromyogram. Wachholder used two needles 2 cm. apart in the substance of the muscle and recorded the action currents with a string galvanometer. With very feeble contractions he found, as we have done, that the individual responses were separated by long intervals and that they formed a definite series with a frequency increasing gradually from a lower limit of about 5 a sec. But with stronger contractions he observed the appearance of a new type of response made up of a continuous train of rapid oscillations (at 200 a sec. or more) becoming gradually larger and larger and finally obscuring the isolated responses of the first type. As a possible explanation of these two types he suggests that the first may represent the initial rhythm of the cortical discharge and the second the rhythm superimposed on the first owing to the stimulation of the muscle receptors, but he is careful to point out the difficulties involved in this view and the exact significance of the two types is left an open question. Again Richter(6) has recorded the electromyogram in normal subjects and those with various nervous diseases, using a diffuse electrode on the head and a pad placed over the muscle and communicating with it by a small hole made through the skin. His records for feeble contractions show the same features as ours, as they give one or more independent series of responses at a low frequency which increases progressively. But he, like Wachholder, seems inclined to attribute the irregular secondary waves which appear in stronger contractions to some different type of nerve discharge less regular than that producing the primary waves. These modern studies may be compared with the original work of Piper and others in which large pad electrodes lead off from the muscle through the skin. By this method there is little or no indication of any change in frequency as the contraction develops. Small, irregular waves are present throughout and it is only in extreme fatigue that a definite change in frequency can be observed.

It will be seen that the detailed structure of the electromyogram becomes clearer and clearer as the electrode system has become better and better adapted for recording the electric change in a few fibres

instead of the average change in the whole muscle. The needles used by Wachholder were 2 cm. apart and so would naturally pick up the responses of a large number of fibres besides those in the line joining the needles. With Richter's electrodes the puncture through the skin covering the muscle will act as a low resistance path leading from a small area on the surface of the muscle to the pad on the skin, and the electric responses will be due mainly to the fibres just below the puncture. With both systems the electric changes in a restricted number of muscle fibres will have the greatest effect on the record, but many other fibres will affect it in a minor degree. These will produce the small oscillations appearing with a strong contraction and the oscillations will be irregular and of high frequency because they are due to very many fibre groups acting independently. There is nothing new in this explanation of the small irregular waves, for it was put forward by Piper in his original work on the electromyogram. Perhaps the best evidence in favour of this view is given by comparing the sound records made with the concentric and with the double wire type of electrode. With the latter the localisation will be sharper, and with strong contractions there is usually one prominent rhythm of fairly high frequency. At present, however, we have not yet succeeded in making good photographic records with this type of electrode.

We conclude that the voluntary contraction in man is maintained, like the reflex contractions in the cat, by a series of nerve impulses which range from 5 to 50 or more a sec. in each nerve fibre, and that the gradation in force is brought about by changes in the discharge frequency in each fibre and also by changes in the number of fibres in action.

(2) *Reflex contractions in the cat. Mechanical stimulation.*

Records made by the same method on various reflexes from the hind limb confirm those already obtained by leading from single nerve fibres, and show that the general character of the discharge in those experiments was not affected by the section of some of the afferent nerve fibres from the muscle. But the ability to record from an intact muscle allows us to study also the reflexes which are elicited by stimulation of end organs in the muscle itself. For instance in the persistent (tonic) contraction of the quadriceps in decerebrate rigidity we have always found a persistent succession of action currents at a frequency of 5–25 a sec. (see Fig. 16 *A*). The discharge may last without interruption for many minutes and the persistence and apparent regularity is such that we have often been deceived into thinking it due to an electrical artefact from a motor or contact breaker in some other part

of the laboratory. Since the series of responses shows no sign of interruption or change in amplitude over long periods, it is unlikely that we

Fig. 16.

Fig. 17.

Fig. 16. *Exp.* 18. Decerebrate cat. Needle in right quadriceps.

A. Persistent "tonic" discharge. Portion of long record. Frequency 9·5 a sec.

B. Stretch reflex produced by flexing leg: beginning of response.

Fig. 17. *Exp.* 19. Decapitate cat. Needle in right tibialis anticus. Beginning of flexion reflex produced by pinching foot. Many fibres seem to be in action.

have to deal with two or more fibre groups acting in rotation, though we have made no observations of the total length of time for which the series may persist and it is quite possible that other fibre groups may come into play at intervals of 10 minutes or more. It is clear that the tonic contraction which suffices to keep the leg extended when the animal is on its back is maintained by only a small fraction of the fibres in the quadriceps, for as a rule the records show only one prominent series of action currents and a change in the position of the needle point in the muscle may alter the size of the responses without bringing in a fresh series. It is our impression that the active fibres are more numerous in the deeper parts of the quadriceps (the crureus etc.), and more numerous in the soleus than in the gastrocnemius, but we have not yet collected enough evidence to be sure of this. The increased contraction produced by manipulating the leg etc. leads to an immediate increase

in the frequency of the persistent impulse discharge and to the appearance of other independent rhythms beginning at a low frequency and increasing rapidly until the record may become completely confused. Records made during the stretch reflex in the quadriceps or gastrocnemius show the same features and are often quite indistinguishable from those made from the human triceps during a progressive voluntary contraction (Fig. 16 *B*).

These records taken in conjunction with those from the divided nerves leave us in no doubt that the persistent contractions of the extensor muscles in decerebrate rigidity are due to low frequency impulse discharges in a fraction of the motor nerve fibres. As regards the frequency range, it is satisfactory to find that our figures agree with those recently obtained by Denny-Brown(7) by an entirely different method[1].

One other point deserves mention for it confirms the observations made in Section I on the changes in the number of fibres in action. In these we found little evidence of an accession of fresh neurones in stronger contractions when we were dealing with the tibialis anticus and peroneus longus in the spinal animal, but definite evidence in the case of the quadriceps in the decerebrate animal. The observations on the muscle action currents agree with this, for the records from the leg muscles in the spinal flexion reflex produced by pinching the foot are quite unlike those from the extensors in the decerebrate animal. Instead of each impulse series standing out sharply with more and more appearing as the contraction develops, we find a much less regular succession of action currents which vary in size and duration and appear to be due to a number of fibre groups responding more or less in phase but at slightly different times. An example of this type of discharge is given in Fig. 17. Since the action currents are nearly always composite in the records from the ankle flexors (spinal) we cannot say how many neurones are in action from moment to moment, but at least there is nothing like the progressive addition of more and more fibre groups which appears in the quadriceps. The differences pointed out by Liddell and Sherrington(9) between the spinal "d'emblée" type of reflex and the recruitment type characteristic of the extensor reflexes are well known and the difference in the character of our records is clearly related to these, but in the hamstring muscles in the spinal animal we have usually found an intermediate type of discharge, beginning with isolated action

[1] The complete account of Denny-Brown's valuable work on the postural reflexes did not appear early enough to allow us to refer to it in detail. In general our conclusions agree so closely with his that we find we have stated them in almost identical terms.

currents and going on very soon to the confused responses due to the simultaneous activity of many fibres near the point of the needle. In man the voluntary contraction of the tibialis anticus gives records showing individual rhythms and the accession of fresh fibres just as clearly as do the extensor muscles in the cat's thigh.

On such questions as the maximum frequency of discharge, the possibility of a rotation of activity, etc. we must reserve judgment until we have more perfect electrodes which can be trusted to pick out the action currents of single fibre groups at the height of a contraction. The concentric type of electrode, however, has one advantage which is not likely to be possessed by systems giving sharper localisation in that it allows us to use electrical stimulation to elicit the reflex contractions. So much of our knowledge of the motor centres has been obtained by this method of stimulation, that we were disappointed to find it impossible to eliminate the stimulus artefact when we were leading from the nerve fibre and correspondingly glad that it could be done without difficulty by leading from the muscle with electrodes of the unipolar type.

(3) *Reflex contractions. Electrical stimulation.*

Records of the action currents in various muscles in response to rhythmic stimulation of afferent nerves were published by Cooper and Adrian(8) in 1924 and a distinction was drawn between the flexion reflex (spinal animal) where the stimulus rhythm is reproduced in the action current record and the crossed extension reflex (decerebrate) where it is usually absent. These differences were found to agree with those shown previously in the muscle tension records by Liddell and Sherrington(9) and subsequent work has confirmed the distinction(10). Hitherto the action currents have been led from an entire muscle, and with this arrangement the electromyogram of the quadriceps in the crossed extension reflex is usually made up of a rapid and irregular series of oscillations of much the same appearance whatever the frequency of the stimuli to the afferent nerve. In some preparations there are larger waves at the same frequency as the stimuli, but throughout the record the rapid irregular discharge is the main feature of the response. On the other hand, in the flexion reflex (spinal animal) the response may consist entirely of large "primary" waves recurring with the frequency of stimulation, though there is sometimes a background of irregular "secondary" waves of high frequency. Each primary wave is obviously due to a more or less synchronous volley of impulses in

many motor fibres evoked by the corresponding volley of impulses in the afferent nerve. The nature of the discharges which produce the rapid irregular waves was more uncertain, but it can be made out by recording from single fibre groups instead of from the entire muscle.

In these experiments the popliteal branch of the sciatic was cut through distally and stimulated by shielded electrodes with break shocks from a coreless coil coupled to a rotating contact breaker of the type described by Cooper and Adrian. The frequency of stimulation was read on an electric speed indicator and was marked on the record by the loud speaker movement carrying a pointer in the eyepiece of the electrometer (see p. 131). The movement was driven by a battery through a circuit interrupted by an independent set of contacts revolving on the same shaft as those in the primary circuit of the induction coil. This arrangement is capable of signalling the stimulation rate up to 200 a sec. or more.

Crossed extension reflex (decerebrate).

As before we were unable to analyse the rhythms of individual fibre groups at the height of the discharge, but the records throughout weak contractions and at the beginning and end of powerful contractions are clear enough. They show one or more rhythms ranging between 10 and 20 a sec., varying with the strength of the stimulus and the period for which it has been in action and usually bearing no relation to the frequency of stimulation. Our clearest records were made from the gastrocnemius and soleus group, probably because these muscles contract less forcibly and the fibre groups which are in action are further apart. A selection from one experiment is given in Fig. 18. Here the stimuli were near threshold value and there was a long latent period. The records show the moment at which the stimulation ends and the beginning of the after discharge. With stimuli at 3, 88 and 228 a sec. the response frequencies are 16, 14 and 18 a sec. With weaker stimuli at 228 a sec. the response frequency is only 11 a sec. There is no break in the rhythm when the stimulus ceases and the degree of regularity throughout the contraction is of the same order as in all the motor discharges already described. Fig. 19 *A* is from another experiment of the same kind made at the end of the after discharge and showing the progressive decline in frequency.

Thus with electrical stimulation of afferent nerves the irregular response of an extensor muscle is built up out of responses in the individual fibre groups which appear not to differ in any way from those produced

Fig. 18. *Exp.* 20. Decerebrate cat. Needle in right gastrocnemius or soleus. Stimulation to left peroneal nerve at various frequencies. Stimuli near threshold strength. In *B*, *C* and *D* the records show the end of stimulation and the beginning of the after discharge.

A.	Stimuli	3 a sec.	Frequency	16 a sec.	
B.	„	88 „	„	14	„
C.	„	228 „	„	18	„
D.	„	228 (weaker).	„	11	„

Fig. 19. *A*. *Exp.* 18. Decerebrate cat. Needle in right quadriceps. End of after discharge of crossed extension reflex produced by stimulating left sciatic at 80 a sec.

B. *Exp.* 20. Decerebrate cat. Needle in right gastrocnemius or soleus. Stimuli at 3 a sec. to left peroneal. Beginning of response, showing double action currents occurring after each stimulus with a shorter and shorter latency. After the third stimulus the usual type of discharge appears.

by the normal stimulation of the sense organs. With weak stimulation the frequency in each fibre group may never exceed 15–20 a sec. There is usually a sharp initial rise to a fixed rate which is maintained during the stimulation period and followed by a more gradual decline. Such changes in rate will naturally alter the force of contraction, but in these reflexes, as in voluntary contractions, the grading must depend mainly on the number of fibres in action.

Except at the beginning of the discharge there has been no indication of the stimulus frequency in these records, but in Exp. 20 with rates of stimulation up to 10 a sec. there is a definite grouping of impulses at the beginning of the response to fairly strong stimuli. An example of this is given in the lower half of Fig. 19. The stimulation

Fig. 20. *Exp.* 19. Decapitate cat. Needle in right tibialis anticus. Stimuli to right peroneal at 6 a sec. Beginning of discharge. The record shows the "primary" response to each stimulus and "secondary" responses after the first three stimuli.

Frequency of secondary discharge after first		stimulus	85 a sec.	
,,	,,	second	,,	75 ,,
,,	,,	third	,,	60 ,,

is at 3 a sec. and two action currents in rapid succession follow each of the first three stimuli, the interval between the stimulus and the double response becoming shorter and shorter (0·081, 0·056 and 0·029 secs.). After the second of these double responses the usual low frequency discharge develops in the usual way and no further double responses are seen after the third. If all the muscle fibre groups behave alike, the contraction would evidently show some indication of the stimulus rhythm during the first second and would then cease to do so. The record is particularly interesting when taken in conjunction with that in Fig. 20 where we see how prominently the stimulus rhythm is represented in the spinal flexion reflex.

Flexion reflex (spinal).

The response of the entire muscle in the flexion reflex is often a single series of large action currents which recur with the frequency of stimulation and can scarcely be distinguished from the action currents

produced by stimulating the motor instead of the sensory nerve. We should naturally expect that the records made with the needle electrode in responses of this type would differ very little, except in the sharpness of the action currents, from those made from the entire muscle, and many of our records from the ankle flexors show nothing more than a series of action currents at the same frequency as the stimulus.

In many preparations, however, the response of the entire muscle shows the rapid, irregular secondary waves as well as the large primary waves, and we were anxious to analyse these into the single fibre discharges. Unfortunately we failed to detect anything but the simple primary wave discharge in all but one preparation, probably because the majority of the neurones discharge at the same rate as the stimuli and only a few contribute to the secondary waves. In one preparation however the response was of unusual type, beginning with a very powerful flexion reverting almost at once to a weaker sustained contraction. This may have been due to a concurrent inhibition set up by the proprioceptors in the leg, for the limb was not fixed and there was no resistance to the movement.

A record of the beginning of the discharge in this experiment is given in Fig. 20. The stimuli occur at 6 a sec. It will be seen that each stimulus is followed by a distinct action current occurring after a brief interval, and that during the first half second there is also a rapid succession of action currents beginning at a frequency of 80 a sec. These are much the same size as the "primary" action currents which follow the stimulus rhythm, and so are probably due to the same fibre group. The rhythm of the rapid discharge is thrown out by each stimulus; in the third period it has fallen to 60 a sec. and it then ceases abruptly, though the small notches on the base line show that much the same thing may be going on for a longer period in other fibre groups. Fig. 20 is typical of many records made from the same preparation and, although the contraction did not follow the usual course, the discharge probably gives a fair representation of the make up of the secondary waves. It appears, then, that each volley of afferent impulses in addition to causing the corresponding volley of motor impulses which make the primary wave, sets up in some of the motor neurones a rhythmic discharge of the same type as that developed in voluntary contractions, and in the reflexes to mechanical stimuli, *i.e.* a discharge which varies in frequency according to the general level of excitation in the motor centres.

Discussion.

The most remarkable point which arises from this paper is that the discharge of a motor nerve cell can scarcely be distinguished from the discharge of a sense organ. In both the frequency of the discharge controls the intensity of the effect which the message produces and is itself controlled by the intensity of excitation. The sense organ may give a series of impulses with a nearer approach to a completely regular rhythm, but in both the frequency varies over much the same range under average conditions of excitation. It must be admitted that the data for the frequencies of the sensory discharge have been obtained partly from cold-blooded and partly from warm-blooded animals, and in only a few cases have we exact information as to the frequency in each nerve fibre. But there is little doubt that a range of 5 to 100 a sec. would cover the frequencies found in a large number of sense organs subjected to a stimulus of normal intensity, just as it covers the normal frequencies of the motor discharge. That the range of frequency should be so much alike is all the more striking when we consider the different structures involved. The only structural factors common to the sense organ and the motor nerve cell appear to be the terminal arborisation which links the axon of the sensory fibre with the cells of the sense organ, and that which invests the nerve cell or forms the junctional zone between its dendrites and the axons of other neurones. The suggestion that we are dealing with some fundamental property of synapses in general is strengthened by the fact that the discharge in the eel's optic nerve fibres seems to be of the same character(11), for in this case the discharge must be generated in the synaptic regions of the retina rather than in the rod and cone layer. Clearly more work must be done before we can accept the view that all nervous messages are of the same general type, but some of the consequences of this view may be pointed out. In the first place it would mean that we must abandon the idea that central inhibition can be explained on the lines suggested by Lucas(12), Forbes(13) and Adrian(14), *i.e.* by the depressant effects produced by high frequency impulse discharge. Various heroic assumptions might overcome the difficulties which would arise, but they are scarcely worth making[1]. We must therefore

[1] Objections to this view have been raised on the score of the lasting inhibitory effect which may follow a single stimulus(15). These objections can be met by the fact that in any case the inhibitory discharge must be produced by a "central process" of the same kind as that which gives the excitatory effect and that this may certainly outlast the stimulus. But on any scheme based on the interference of trains of impulses

revert to the view that two qualitatively different processes can occur in the synapses to account for the inhibitory and the excitatory effect. A scheme on these lines has been worked out in detail by Sherrington(16), and it is there suggested that excitation of the motor neurones by the afferent impulses is due to the liberation of a specific exciting substance in the synaptic regions. The work of Lewis(17) on the control of the skin vessels by impulses reaching them through the collaterals of sensory fibres, and that of Dale and Gasser(18) on the contractions produced in denervated muscle by stimulation of the sensory nerve and by drugs of the acetyl choline class, suggest very strongly that the terminations of sensory fibres may produce substances which have a stimulating effect on certain tissues, and the production of NH_3 during the activity of medullated nerve shows that the passage of an impulse into the spreading central terminations of an afferent fibre might liberate substances of considerable activity. It is, however, quite possible that all the discharges of the motor neurones are not brought about by the same mechanism. In the spinal flexion reflex, for instance, each volley of afferent impulses seems to be directly transmitted to the motor neurones, and there is little reason to suppose that we have to deal with a process differing greatly from that involved in the conduction of an impulse from one section of a nerve fibre to the next. But the production of the secondary waves (Fig. 20) demands a mechanism of the type operating in the crossed extension reflex where direct transmission does not occur, and here the cause of the discharge may well be a more lasting excitatory state produced in the synaptic region as a result of the passage of each impulse. Sherrington has pointed out how the latent period, rate of rise and length of after discharge in the crossed extension reflex depend on the strength of stimulation, *i.e.* on the concentration of impulses arriving at the synaptic regions. Again, in the optic nerve discharge the latent period depends on the number of receptors in action as well as on the intensity of the stimulus, and it seems as though each impulse which enters the synaptic zone within a given time adds its quota to the excitatory effect—as we should expect it to do if the effect depends on the presence of something liberated by the impulse and not immediately destroyed.

we have to face the difficulty that in an intense excitation, where the central paths are discharging at fairly high frequencies, inhibition should be brought about much more easily than in a feeble excitation where the frequency is lower. In fact if intensity of excitatory effect depends on frequency, the greater the excitation the smaller will be the degree of inhibition needed to extinguish it.

Whatever the excitatory process may be, we can visualise its operation in the motor centres by the diagram given in Fig. 21. This is intended to represent the development of a contraction such as the crossed extension reflex in the quadriceps. The motor neurones are indicated by the vertical lines and the numbers on these lines represent the frequency of discharge from the neurone when the excitation reaches a

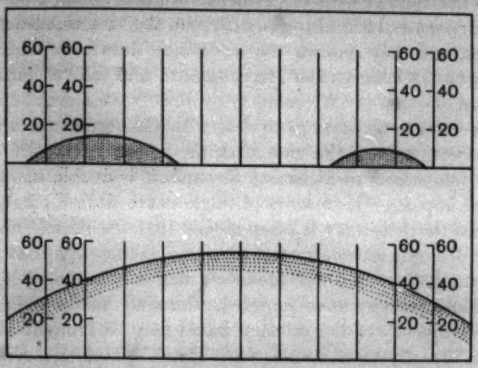

Fig. 21. Development of excitation process in a group of neurones (see text).

particular level. At the beginning of the contraction there are a few isolated centres of excitation producing a low frequency discharge in a few neurones; as more sensory impulses arrive the level of excitation rises and spreads over the motor centre, giving a high frequency discharge in many nerve fibres. This diagram applies only to the recruitment type of reflex where the connection between afferent and efferent neurones is indirect. As we have seen, in the spinal flexion reflexes a given afferent nerve or group of receptors is much more closely linked with a given group of motor neurones. When an afferent nerve is stimulated rhythmically the motor neurones discharge with the same rhythm: when receptors are stimulated the frequency of the motor discharge varies with the strength of the stimulus, *i.e.* with the frequency in the afferent fibres, but a high frequency discharge from a given group of receptors shows no tendency to activate more motor neurones than one of low frequency. With the mechanical stimuli which we have used only a fraction of the muscle can have been active; that fraction remained constant and the contractions were graded entirely by the frequency, because we varied the

intensity of the stimulus rather than the area covered by it. Graded electrical stimulation of an afferent nerve must produce just the reverse of this, for here (if we neglect the secondary waves) the frequency of the motor discharge is fixed by the frequency of stimulation, but an increase in the stimulus will excite more afferent fibres and so will activate more motor neurones. It will be seen, therefore, that our results do not conflict with the evidence brought forward by Cooper, Denny-Brown and Sherrington (19) as to the "fractional activation" of muscles, and they confirm the distinction drawn by Liddell and Sherrington (9) between the "recruitment" and the "d'emblée" type of response.

We have made no attempt to deal with the arguments which have appeared in support of the view that the motor nerve fibre may discharge at frequencies so high that the muscle is unable to respond to each nerve impulse. The records of single nerve fibre discharges show that in fact the frequency is often so low that the muscle fibres must fail to give a smooth contraction, but we cannot say that maximal sensory stimulation may not produce reflex discharges of higher frequency than those we have recorded. Certainly in the spinal flexion reflex the frequency of the primary waves may be brought well above 100 a sec. by rhythmic stimulation. But the fact that the average frequency with a fairly strong contraction is round about 50 a sec. in our nerve fibre records makes it difficult to accept the most plausible line of evidence in favour of very high frequencies as a normal occurrence in certain reflexes. This evidence is derived from the alcohol block method used by Forbes and Olmsted (20) and Liddell and Olmsted (21) and based on the view that alcohol will block or at least diminish the frequency of a train of high frequency impulses whilst leaving a lower frequency untouched. The fact that alcohol applied to the motor nerve causes an extinction of certain reflexes although the nerve can still conduct when stimulated electrically is held to indicate that the reflex discharge must have been extinguished in virtue of its high frequency, and for this to occur the impulses must follow one another at intervals which Forbes and Olmsted reckon as certainly no more than 3·5 and probably no more than 3σ. But it is clear that an alcohol block which only comes into action when the frequency of the discharge exceeds 300 a sec. would not have the least effect on any of the discharges recorded in our experiments on single nerve fibres, where the frequency has never risen above 100. The failure of the reflexes under the alcohol block must be due to some other cause than the high frequency of the

discharge, and if this be admitted it becomes doubtful if the method can really furnish reliable information about the reflex frequency.

An important point which must be left untouched concerns the degree of synchronous activity in the neurones carrying out a particular reflex. This was discussed in Part I, and for the present we have little to add, as we have been mainly concerned with the individual neurone.

All these questions relate to the mechanism of the neurone, but there are several points of interest relating to the muscular contraction and arising from the fact that, except in powerful contractions, the response of the individual fibres will be an incomplete tetanus or often little more than a succession of twitches. A contraction sustained in this way may need a greater expenditure of energy than one due to a complete tetanus in fewer fibres, but it may have the great advantage of preventing the accumulation of metabolites which causes the early fatigue of a complete tetanus. This would be due in part to the long time elapsing between each excitation, but the removal of waste products might also be favoured by the improved blood supply. It is at least probable that the flow of blood through a steadily contracted muscle will be much smaller than through one where the different fibre groups are in continual movement owing to the intermittent contractile waves set up by a low frequency discharge. Thus the type of discharge which we find in the postural reflexes seems excellently adapted for maintaining a moderate degree of contraction for long periods without fatigue.

Summary.

Records have been made of the reflex impulse discharges in individual nerve fibres supplying various muscles in the hind limb of the cat. The method was that previously used for recording discharges in the nerve fibres of the phrenic. The reflexes (spinal flexion and decerebrate crossed extension) were produced by mechanical stimulation (pinching the foot), and the discharges were recorded in nerve fibres supplying the peroneus longus and tibialis anticus (flexion) and the vastus medialis and lateralis (extension). Confirmatory experiments were made by leading from restricted groups of muscle fibres by electrodes of very small dimensions. The action currents were recorded by a three valve amplifier and capillary electrometer, and much information was gained by converting the action currents into sound waves by means of a second power amplifier and a loud speaker. The main results are as follows:

(1) Flexion reflex (spinal). In the nerve fibres to the peroneus longus the discharge has been a series of impulses recurring fairly regularly

at a frequency which varies from 5 to 25 a sec. The frequency increases as the contraction develops. In the fibres to the tibialis anticus the discharge is of the same type but the frequency may reach 45 a sec. With mechanical stimulation of the foot the grading of the contraction appears to be due mainly to this change in frequency, for there is little evidence of changes in the number of neurones in action.

(2) Extension reflexes (decerebrate). In the nerve fibres to the quadriceps there are often persistent discharges ranging from 10 or less to 25 a sec., when the postural tone of the muscle is well marked. During a crossed extension or an extension due to stimulation of the labyrinths the discharge rises to frequencies which may be as high as 90 a sec., and there is evidence that many fresh neurones come into play as the contraction develops.

(3) Records of muscle action currents made by concentric needle electrodes show the discharge of groups of muscle fibres innervated by single neurones, and these agree with the discharges recorded in the single nerve fibres. At present the method can only be used with certainty during weak contractions where the active fibre groups are some distance apart, but in those we find the same low frequency discharges and the same increase in frequency and in the number of fibres in action as the contraction increases.

(4) During voluntary contraction of the triceps in man the impulse discharges are of the same type as those in the cat's quadriceps. The electromyogram of the entire muscle is compounded of the rhythmic discharges in the different groups of muscle fibres. These vary in frequency with the strength of the contraction, and the irregular high frequency oscillations in the electromyogram are due to fibre groups responding out of phase with one another.

(5) The persistent tonic contraction of the quadriceps in decerebrate rigidity is maintained by discharges at a frequency of about 5–25 a sec. from some of the neurones. During the stretch reflex the frequency increases and more neurones come into play.

(6) When the crossed extension reflex is elicited by rhythmic electrical stimulation to a sensory nerve, the frequency of the discharge usually bears no relation to the frequency of the stimuli, but increases with the contraction from a lower limit of 5–10 a sec.

(7) In the spinal flexion reflex with electrical stimulation each volley of afferent impulses produces a corresponding response in the motor neurone. In the intervals between these responses there may be a rhythmic discharge of varying frequency.

(8) The likeness between the impulse discharge from a sense organ and that from a motor nerve cell suggests that both are due to some property common to axon or dendrite terminations in general.

(9) The low frequency of discharge in the motor nerve fibres during weak contractions may be of importance in preventing the accumulation of metabolites within the muscle fibres and in assisting the blood flow through the muscle.

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THE REVERSIBLE LOSS OF EXCITABILITY IN ISOLATED AMPHIBIAN VOLUNTARY MUSCLE[1].

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A. Introduction.

In the spring of 1928 an attempt was made to confirm, by direct chemical estimation, the very low values obtained by Stella(1) by a diffusion method for the inorganic phosphate content of resting frog's muscle. It was hoped to secure a really resting condition by employing very thin muscles and suspending them in moist oxygen for some hours. The slowness of diffusion on the one hand and the consumption of oxygen in the resting metabolism on the other (see (2)) may prevent the inner region of a thick muscle from being adequately oxygenated, so that resting conditions may not really exist there: when, however, a thin sartorius muscle of *Rana temp.* or of *Rana esc.* is suspended at rest in pure oxygen the whole of its interior may be regarded as being adequately supplied with that gas. Experiments, therefore, were made in which frogs' sartorii were suspended in moist oxygen for one or more hours at room temperature. The temperature was then reduced to 0° C. approximately and, after an interval sufficient to allow them to become cold throughout, the muscles were ground up in ice-cold trichloroacetic acid. The extract was analysed, with slight modifications[4], by the method of Eggleton and Eggleton(3), with whom these initial experiments were made.

Values for the inorganic phosphate content varying from 10 to 20 mg. p.c. were obtained, with correspondingly high values for the phosphagen phosphate, instead of the previous values of 20 to 30 mg. p.c. The values obtained by Stella were about 8 mg. p.c. for muscles suspended in oxygenated Ringer's solution.

[1] A preliminary account of these experiments was read to the Physiological Society at Oxford on July 14th, 1928, and a more complete account at the meeting on Jan. 19th, 1929.

[2] Rockefeller Foundation Fellow. [3] Working for the Medical Research Council.

[4] For an improved method of analysis, incorporating these and other modifications, see Eggleton and Eggleton (in the press).

Fletcher and Hopkins(4) have shown, and many subsequent observers have confirmed, that many extraction processes can give fictitiously high values to the lactic acid content of muscle, owing to the "stimulation" which results from the grinding up process. It appears that with phosphagen and inorganic phosphate determinations also, the utmost care must be exercised to avoid this source of error.

During the course of the preliminary oxygen treatment at room temperature it was often noticed that the muscles became inexcitable. Such muscles at first were discarded for the above work, it being suspected that they were in poor condition, as the frogs had been for some time in the laboratory. It soon became evident, however, that this behaviour was the rule rather than the exception, and analyses were made to determine whether the muscles which had become inexcitable showed the characteristic chemical properties of fatigue or rigor. In all cases the phosphagen content of an inexcitable muscle was found to be comparable with that of a resting excitable one, and since Eggleton and Eggleton have shown that in rigor all, and in fatigue most of, the phosphagen is broken down, it was clear that the phenomenon in question could not be attributed to "fatigue" or "death."

B. The action of oxygen.

If a freshly excised frog's sartorius (dissected out, unless otherwise stated, without the use of Ringer's solution) be suspended in water-saturated oxygen there is a progressive decrease in the inorganic phosphate and free creatine and an increase in phosphagen (estimated by the method of Eggleton and Eggleton) and in combined creatine (estimated by the method of Dulière(5)). This process is attended by, if it be not actually a part of, the oxidative removal of lactic acid, and hence an improvement of the state of the muscle might be expected. Sometimes, and at first, this may appear to be the case, but, after a period, usually of an hour or two (varying between 30 minutes and a day, although rarely longer than 5 hours), the muscle ceases to respond to stimulation, although it still conducts electricity as can be shown by including an excitable muscle in series with the inexcitable one.

A muscle which has just become inexcitable to a stimulus previously maximal will sometimes respond to an increased stimulus, but after a further interval in the gas, the muscle fails to respond at all. The phenomenon appears to be unaffected by the type of exciting current used: direct current (2–4 volts) gave similar results. In general, a one-second tetanus was employed for testing the excitability, the strength

being adjusted initially to be just maximal and the stimulus applied by electrodes placed directly on the muscle.

The amount of phosphagen in these muscles may be very high, as might be expected from the oxygen treatment, and the inexcitability may be maintained for at least 20 hours in oxygen, without affecting the phosphagen content. Prolonged stimulation of an inexcitable muscle (for example, 300 one-second tetani at three-second intervals, sufficient under ordinary circumstances to destroy all the phosphagen) is entirely without effect on the phosphagen content. Hence it appears that the breakdown of phosphagen is conditioned either by the excitability or by the contractility of the muscle, and not by the application of an electrical stimulus as such.

C. The use of gases other than oxygen.

Since the oxygen treatment involves the removal of traces of lactic acid initially present, and since in a thin muscle carbon dioxide would be expected to diffuse out, alkalinity progressively increasing might be supposed to be the cause of the inexcitable state which supervenes. That this is not the case is shown by the appearance of the phenomenon when air, commercial nitrogen, or 5 p.c. carbon dioxide in air is used instead of oxygen. Prof. A. V. Hill has verified the fact that a sartorius muscle becomes inexcitable in moist nitrogen which has been purified from oxygen by passing it slowly over heated copper. Since in these cases the alteration in phosphagen content is comparable with that for excitable muscles (Eggleton and Eggleton(6) have shown that an increase occurs in aerobic, and a decrease in anaerobic conditions) changes in phosphagen associated with the respective actions of these gases are not connected with the production of the inexcitable state.

D. The water content of the muscle.

In the earlier experiments the stream of gas, which was maintained for the whole duration of the treatment, was bubbled through water or Ringer's solution; it might not have been completely saturated and hence might have been able to remove a small amount of water from the muscle, though, in fact, the latter appeared to be quite wet at the end of the treatment. In later experiments use was made of a bottle containing oxygen and about 50 c.c. of Ringer's solution, completely sealed up; the muscles were suspended in the gas above the solution by silver wires running through the stoppers and were tested without opening the bottle. Others were treated with oxygen which had been

completely saturated with water by passing through a cylinder packed with wet cotton wool and then through Ringer's solution. It appeared in some of the earlier experiments of this type that the time required for the onset of inexcitability had been increased by the change in conditions, but further controlled experiments, in which two muscles of the same frog were used, showed that in fact no real difference existed.

The independence of the phenomenon in question of the water content of the muscle was finally proved by the success of experiments made with mercury and with thick paraffin oil. Sartorii immersed for 5 minutes in Ringer's solution were surrounded with mercury or paraffin. In the latter case a control muscle was inserted in the electrical test circuit to check the possibility of the muscle being insulated from the silver electrodes and hence appearing inexcitable when it was really unstimulated. In the case of the mercury experiments the test for excitability was made by removing the mercury from the muscle by tilting the tube containing it. Contact with the muscle was established by iron wires tied to it. In both cases the muscles became inexcitable in about an hour. Hill and Hartree ((7), p. 93) noticed in 1921 that liquid paraffin "acts unfavourably on the condition of the muscle."

E. The restoration of excitability in Ringer's solution.

It is a matter of common knowledge with those who have worked with isolated muscles that a failing muscle may be revived by immersing it in Ringer's solution. Thus, for example, Hill and Hartree ((7), p. 92) record that it is best, "for the purpose of maintaining the condition of the muscle, to fill the (thermopile) chamber with Ringer's solution" and to replace this by oxygen only when the actual measurements of heat production are to be made. In the type of non-irritability examined by Foster and Moyle(8), to which further reference will be made below, immersion in Ringer's solution will restore the irritability of muscles previously entirely unaffected by a stimulus. In the present case the effect of immersion in Ringer's fluid is striking and may be very rapid. The solution employed was of the composition: NaCl 0·65 p.c., KCl 0·014 p.c., CaCl 0·012 p.c. The solution used need contain neither phosphate, nor bicarbonate, nor oxygen. A resting muscle, which has become inexcitable by standing in a gas, may return to a condition in which the tension developed in response to a stimulus may be as large as, or even larger than, it was initially. No permanent damage appears to result from a period of inexcitability. Muscles rendered inexcitable by immersion in paraffin are revived without any treatment designed

to remove the oil, which seems to have no effect on the restorative action of the solution.

It may be recalled that Gottschalk(9) found that nerves rendered inexcitable by deprival of oxygen can be restored in part, either by readmission of oxygen, or by washing with oxygen-free Ringer's solution, but that complete recovery requires both oxygen and washing.

F. The restoration of excitability by solutions other than Ringer's.

The solutions capable of restoring excitability to an inexcitable muscle may be divided into two classes: (*a*) those in which a muscle progressively regains its excitability, and (*b*) those in which a muscle at first regains a certain degree of excitability which it loses completely on further immersion. A muscle allowed to remain inexcitable for long may not be revived at all by solutions (*b*).

Effect of various solutions on inexcitable muscles.

Permanent recovery	Temporary recovery	No recovery	Subsequent action of Ringer's solution
Ringer's solution			
Ca-free ditto			
Isotonic NaCl			
Isotonic $NaNO_3$ (one exp. only)			
Ringer's solution with 0·05 p.c. or 0·1 p.c. NH_4Cl*			
50/50 p.c. mixture of Ringer's solution and isotonic glycerol			
	Isotonic glucose (one exp. only)		Complete recovery in approx. 15 mins.
	Isotonic Na_2SO_4		Ditto
	Isotonic LiCl (one exp. only)		Ditto
	25/75 p.c. mixture of Ringer's solution and isotonic glycerol†		
		Isotonic glucose (four cases)	Ditto
		Isotonic LiCl (three cases)	Ditto
		Isotonic $NaHCO_3$	Ditto
		Distilled water	Ditto
		Isotonic NH_4Cl	No recovery

* This was used to test the suggestion that inexcitability might be due to the accumulation of traces of ammonia.

† Subsequent recovery in a 50/50 p.c. mixture of Ringer's solution and isotonic glycerol.

G. Permanence of excitability and inexcitability.

While a muscle will remain inexcitable for 20 hours if left in oxygen and will then recover if placed in Ringer's solution, a long preliminary treatment with this solution appears to render a muscle permanently excitable in the sense of the present experiments. A. V. Hill (10), p. 134) has found that for myothermic experiments muscles "behave best and survive longest if, after dissection, they are soaked for some time, one to three hours, in Ringer's solution before stimulation is begun." In our experiments it appears that a series of changes from the excitable to the inexcitable state, and *vice versa*, with treatment by Ringer's solution, is possible only until the sum of the times of immersion in Ringer's fluid has reached a critical value. Prof. A. V. Hill informs us (see also (10), p. 157) that a muscle treated with Ringer's solution for a period, sufficient in ordinary circumstances to ensure its permanent excitability, may become inexcitable when allowed to recover in oxygen after having been stimulated to exhaustion in nitrogen; this inexcitability can, as in our experiments, be removed by treatment with Ringer's solution.

H. Temperature.

Results obtained early in July appeared to indicate that inexcitability could not supervene at temperatures above 25° C. Sartórii of *Rana esc.* (Hungarian) did not become inexcitable until the temperature was reduced to below 20° C. Attempts to confirm this in September failed. In one case a frog was kept at 27° C. overnight before killing. The muscle was rapidly excised and placed in oxygen at 27° C.: it became inexcitable in about one hour. Raising the temperature of inexcitable muscles from 20° to 27° C. was without effect. The divergences found are probably to be attributed to seasonal and other variations in the muscles employed.

I. Types of muscles and frogs.

During the course of the work the gastrocnemii and sartorii of *Rana temp.* and *esc.* (English, Dutch and Hungarian) have been employed and have shown the same phenomenon of inexcitability. No regular difference between the species can be stated, though occasional differences occur. In general sartorii become inexcitable far more regularly and quickly than do gastrocnemii. In some cases this has been particularly striking; sartorii after careful dissection have become inexcitable

in from 20 minutes (the shortest time noted) to 2 hours, while gastrocnemii from the same frog have shown signs of bacterial decomposition before the onset of inexcitability. Similarly the skinned legs of a frog from which sartorii have been removed will remain excitable for hours after the dissected muscles have become inexcitable, even when they have been partly dried by remaining on the bench without precautions. Gastrocnemii may show all the phenomena described; more than 20 definite cases have been recorded. The occurrence however is not regular as in the case of sartorii, and inexcitability, when it occurs, may take longer to come on. Only sartorii are now being used for this work. Removal from a normal environment in contact with other muscles seems to have some effect on the phenomenon. That the sartorii are not seriously injured by the dissection is shown by the fact that after treatment with Ringer's solution they may remain excitable and in good condition for days. The difference between individual frogs is particularly marked. Two frogs which appear to be equally active and which are of the same size may provide muscles which will differ several-fold in the time taken to become inexcitable (*e.g.* 40 minutes and 5 hours respectively). Many of the frogs used have been extremely fresh and active; the effect cannot be attributed to poor condition in the animals.

J. Tortoise muscles and crab nerves.

The biceps cruris muscle of the African land tortoise rapidly became inexcitable (September) and behaved in a manner closely resembling that of frog's muscle. Furusawa (private communication) has recently found that the limb nerve of *Maia*, which is non-medullated, becomes inexcitable if exposed for a very short time to moist air immediately after removal from the animal. It may be recovered entirely by immersion in sea water for 30 minutes or less, and will then remain excitable for a long period. Isotonic glucose will not restore it.

K. The time relations of excitation.

Preliminary experiments indicate that the value of the chronaxie increases gradually with the onset of inexcitability. There is a considerable rise in the value of the rheobase. It is of interest to note that Lucas (III), p. 471) found a most striking alteration in the time value of excitation, in a sartorius muscle immersed in Ringer's fluid, the change reaching completion in a few hours. The chronaxie decreased enormously as the result of soaking, and the minimal current gradient largely increased. Lucas attributed this to the presence of free calcium

ions in the Ringer's fluid. It might equally well be due to the washing away of excess of potassium ions (see below). The similarity in the times of immersion required (*a*) to produce the effect described by Lucas, and (*b*) to render a sartorius permanently excitable in the present sense, suggests that the loss and recovery of excitability described here are due to changes in the excitatory (as distinguished from the contractile) process, and may, perhaps, be attributed to progressive alterations in the concentrations of Ca or K ions in the muscle fibres or the spaces between them.

L. The production of reversible inexcitability by solutions.

Cooke(12) has shown that inexcitability may be obtained by immersion in hypertonic solutions. This fact was confirmed. A solution of double the concentration mentioned above, and a Ringer's fluid having twice the usual concentration of sodium chloride, caused inexcitability in several muscles tested. Immersion for 10 minutes in normal Ringer's solution restored excitability in all cases.

It is well known that an increase of potassium or calcium in Ringer's solution may lead to the onset of inexcitability in a muscle immersed in it. See, for example, Sereni ((13), p. 13), where full references can be found. According to Sereni a muscle may become inexcitable when immersed in Ringer's solution containing rather more than three times the usual potassium concentration, sometimes in a solution containing only $2\frac{1}{2}$ times the usual concentration. Recovery is possible in such cases by washing in ordinary Ringer's fluid. Higher concentrations of potassium (0·2 p.c. KCl) may cause contracture of the muscle. It seems conceivable that the onset of the inexcitable state may be due to small quantities of potassium diffusing out from the interior of the fibres to the interspaces[1] between them, a possibility that is borne out by the experiments of Ernst and Scheffer(14) who found that potassium does, in fact, leave an isolated muscle perfused with Ringer's solution.

Prof. Lovatt Evans, who called our attention to this possibility, has made experiments (unpublished) in which a state of tonus occurring in a smooth muscle suspended in oxygen could be abolished by immersing the muscle in oxygenated Ringer's solution. As is well known, addition of a small quantity of a potassium salt will cause a contracture in many types of plain muscle.

[1] The effect of high K values in the Ringer's fluid must be due to changes produced in these interspaces: for the K concentration inside the fibres is far higher than in the Ringer's solution which produces the inexcitability.

M. Discussion.

Reversible inexcitability, therefore, may rapidly be induced in frogs' muscles by dissecting them, preferably without the use of Ringer's fluid or with minimal use thereof, and placing them in a gas, or in an environment, such as mercury or paraffin, which is free from electrolytes. Sartorius muscles, perhaps because they have a greater surface per unit volume, perhaps because they require more dissection, become inexcitable more quickly and more regularly than do gastrocnemii. Hermann(15) in 1867 showed that while gastrocnemii retain their irritability better in oxygen than in air, the reverse was the case for sartorii. He advanced a surface theory to account for this. Fletcher(16) in 1902 found that loss of irritability is delayed by oxygen in both cases. The apparent contradiction between the two results may be explained on the grounds that the loss of irritability in the experiments of Hermann was a reversible one in the sense of the present work, while the maintenance of irritability found by Fletcher referred not to excitability as such, but to the oxidative removal of lactic acid and to the other reactions which occur in a muscle when its life and normal condition are maintained by oxygen. Owing to the very great differences which exist between individual frogs, and to the possibility that Hermann did not use any saline solution when dissecting, it is easy to see how one set of muscles might not become inexcitable while the others did.

Reference has already been made to the observations of Foster and Moyle(8). A variety of experiments conducted in the Biochemical Laboratory at Cambridge (see *e.g.* (8), (17), (18)) have shown that when frogs' muscles (gastrocnemii, or the whole musculature of the hind limbs) are kept at 0° C. in oxygen or air, they become non-irritable after a period varying from 1 to 2 weeks. At 0° C. the rate of metabolism is so reduced that diffusion of oxygen is fast enough to prevent any increase in the concentration of lactic acid. The condition of non-irritability in question could thus be sharply distinguished from that associated with the familiar events which, when oxygen is deficient, lead gradually to rigor and death. In these experiments at Cambridge, all at 0° C. on the whole legs or the gastrocnemii of English fen temporaria frogs, in no single case (so Sir F. G. Hopkins informs us) has non-irritability been observed to occur in less than 5 to 6 days. When a condition of non-irritability is established, the lactic acid content is that of the "resting minimum" of fresh muscles (Foster and Moyle(8), Lascelles, quoted by Thomson(17)).

If such non-irritable muscles be transferred to an atmosphere of nitrogen the lactic acid increases normally. They display normally the phenomena of heat rigor, and, under the influence of chloroform or toluene they behave like fresh muscles. They show a normal increase in lactic acid as the result of injury or disintegration.

The effect of ions in reducing the concentration of "inorganic"[1] phosphate is the same in preparations of these non-irritable muscles as in preparations from fresh muscles (Edsall(18)).

On the other hand, the osmotic properties of the non-irritable muscles studied at Cambridge are abnormal; a solution which is isotonic to the normal muscles is hypertonic to a non-irritable one. The internal resistance after 2 hours' washing with isotonic cane sugar solution is increased (Thomson(17)).

A non-irritable sartorius was found to give no action current, but a definite, though diminished, injury current (Foster and Moyle(8)).

Such muscles, even when their non-irritability has been long maintained, are readily restored by immersion in Ringer's solution.

The fundamental difference, therefore, between the experiments in the Biochemical Laboratory at Cambridge and ours, lies in the time required for the production of inexcitability; in those, of the order of a week, in ours of the order of an hour. It will have been noticed that Foster and Moyle employed mostly the complete legs of English frogs, which we have never observed to become reversibly inexcitable; they employed also the gastrocnemii of English frogs, which rarely have become inexcitable in our experiments. The rapid onset of reversible inexcitability at room temperature is most obvious in the thin dissected sartorius. Whether the Cambridge phenomena and those we have described are essentially the same only future study can decide. They are alike in that the chemical state of the inexcitable muscle is in either case analogous to that of a resting excitable one.

One of us (W. D.) has shown(19) by perfusion of the hind limbs of *Rana temp.* with calcium-free Ringer's solution that inexcitability may occur without the phosphagen content being affected. While this case differs from the others in that calcium has been removed and that therefore a chemical change is involved, it resembles them in that from the chemical point of view the muscle is otherwise in a resting condition. The criterion of rest in the experiments of Foster and Moyle was the lactic acid content, in the present work the phosphagen content, checked

[1] This included the "phosphagen" phosphate.

by the ability of the muscle to perform work after recovery of its excitability.

When a muscle is removed from its normal environment it is conceivable that changes may take place in the membranes surrounding the muscle fibres, or in the relative volumes of fibres and interspaces. Hopkins(20), in discussing the work of Foster and Moyle, suggested that a dislocation between stimulus and energy discharge had occurred, and that associations between colloids and electrolytes may have assumed increased stability in the time required for the onset of non-irritability; in either case changes might result in the relative concentrations, inside and outside the fibre, of potassium, calcium or other substance. One outstanding difficulty in this respect is that a muscle which has been soaked in Ringer's solution for an hour or more may remain permanently able to withstand contact with an environment free from electrolytes. Gottschalk(9) working with the sciatic nerves of frogs found that the time required for a nerve to become non-conducting when deprived of oxygen was lengthened by preliminary treatment with hypotonic sodium chloride, and decreased with hypertonic sodium chloride. He suggested that the reason for this was a change in metabolic rate caused by alterations in the water content and not by changes in specific electrolytes.

No explanation can at present be advanced of why a sartorius removed from a leg will become inexcitable while the leg remains excitable. On the whole we are inclined to attempt a further investigation on the lines indicated by Prof. Lovatt Evans' suggestion of the importance of potassium ions. Owing either to leakage outwards of potassium, or to osmotic diminution of the volume of the interspaces, potassium ions become concentrated in the interspaces, and, since immersion in Ringer's solution containing a small excess of potassium causes inexcitability to appear, such an increase in the potassium concentration of the interspaces might be expected to have the same result. The phenomenon, therefore, would become one related to excitability as such, a subject which has been widely investigated by those who have studied the excitation process in muscle and nerve. The rapid recovery of excitability in Ringer's fluid of a muscle which has just become inexcitable reminds one of a diffusion process in that it is rapid at first and then more slow as time goes on, and it may be that the recovery is due simply to the diffusion of potassium ions from the interspaces into the Ringer's solution[1].

[1] Preliminary experiments show that excitable sartorius muscles will become inexcitable in about an hour if placed in Ringer's solution containing four times the normal

The fundamental difficulty of this general explanation is that it is not clear how a preliminary treatment with Ringer's solution prevents any subsequent accumulation of potassium in the interspaces when the muscle is suspended in a gas. Possibly the permeability of the membranes of the fibres is affected by the "shock" of dissection, however careful, and only returns gradually to its normal condition after prolonged exposure to a normal environment of electrolytes.

In later work it is proposed to investigate further the changes in chronaxie associated with the onset and removal of inexcitability, which may throw some light on the excitation process, and also to determine whether a muscle which does not respond to electrical stimulus will respond to chemical treatment. A resting excitable muscle if ground up slowly in 4 p.c. trichloroacetic acid gives an extract, which, on analysis, resembles that obtained from a fatigued muscle. It is proposed to treat inexcitable muscles in the same manner. Preliminary observations of this kind suggest that no difference will be found between muscles of the two types. In this connection Mines and Dale(21) found that the skeletal muscles of the ray, when made non-irritable to electrical stimulation by treatment with dilute ether, respond to the application of acid, alkali and salt solutions.

Summary.

1. In the estimation of phosphagen and inorganic phosphate in muscle care must be taken to avoid the "stimulus" provided by dissection and extraction. A thin muscle suspended at rest in oxygen for some time, and ground up in the cold, gives lower values of inorganic phosphate, and higher values of phosphagen, than ordinary "resting" muscle.

2. Isolated frogs' sartorius muscles, dissected without the use of Ringer's solution, and suspended at room temperature in moist oxygen, nitrogen, air, air with 5 p.c. CO_2, paraffin oil or mercury, rapidly become

potassium content, and that muscles which have become spontaneously inexcitable, or those rendered inexcitable by the above treatment, will recover their excitability if placed in Ringer's solution containing three times the normal amount: spontaneously inexcitable muscles, placed in Ringer's solution containing four times the normal amount of potassium, remain inexcitable and do not show even a temporary recovery of excitability.

The inexcitable condition induced, by immersion in carbon dioxide, in a muscle previously soaked in Ringer's solution, remains after the carbon dioxide has been replaced by oxygen: the muscle, however, may then be made excitable by soaking in Ringer's solution.

reversibly inexcitable. Gastrocnemius muscles may do the same, but the occurrence is neither frequent nor regular.

3. Such inexcitable muscles do not appear to differ chemically from excitable ones.

4. Excitability may be rapidly restored by immersion in Ringer's fluid, or in various solutions. The period of inexcitability may be extended over many hours without affecting ultimate recovery on treating with Ringer's fluid.

5. The onset of inexcitability may be prevented by previous prolonged soaking in Ringer's fluid. Onset and recovery may be repeated several times, until the total period of the immersion reaches a critical value, after which the muscle will remain permanently excitable in the sense of these experiments.

6. The phenomena are not due to drying, to changes in the total water content, to temperature, to the condition of the animals, or to gross injury.

7. Leg muscles of the African land tortoise and limb nerves of the spider crab show similar reversible effects.

8. The chronaxie increases gradually with the onset of the inexcitable state; according to Lucas it decreases during soaking in Ringer's fluid.

9. Reversible inexcitability is readily produced by soaking a muscle in Ringer's solution containing three times the normal potassium ion concentration (Sereni). Potassium is known to escape from the fibres of a perfused muscle (Ernst and Scheffer). It is suggested that an increase in the concentration of the potassium ions in the interspaces between the fibres may be the basis of the phenomenon described. The washing away of these ions would, in that case, be the cause of the restoration.

10. A comparison is made of the present results with those obtained in the Biochemical Laboratory at Cambridge. It is not certain that the two sets of phenomena are the same. They are similar in that no chemical differences have been found between excitable and inexcitable muscles of either type. They are different in the times involved in the onset of the inexcitable state (an hour and a week respectively).

In conclusion we desire to express our gratitude to Prof. Lovatt Evans and Prof. A. V. Hill for their advice and criticism during the progress of the work.

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NOTE ON THE CENTRAL NERVOUS SYSTEM OF THE CRAYFISH.

By W. B. HARDY

For the purpose of this note the freshwater crayfish (*Astacus fluviatilis*) is a small edition of the lobster about three inches long whose central nervous system consists of a supraœsophageal ganglion connected by circumoral commissures with the post-œsophageal ganglia. These two together constitute the brain[1].

From the brain there stretches backwards on the ventral aspect of the animal a ganglionated cord with one ganglion in each segment. The cord is enclosed in a tough sheath which forms the wall of a blood sinus within which the cord is suspended. The sheath gives to the cord the appearance of a delicate white thread with swellings at intervals, but when the sheath is opened the cord is seen to consist of two separate cords which are joined by transverse commissures at each ganglion (4).

Operations on the cord are not impossible but difficult owing to its small size, the presence of a longitudinal artery attached below to the sheath, and the relative toughness of the sheath.

The nerve cells of the post-œsophageal cord need special mention. They hang like berries in the blood stream below each ganglion and the single process of each cell forms a T-shaped junction with its nerve fibres. The longitudinal commissures connecting these ganglia are entirely free from nerve cells. They correspond wholly to the white matter of the spinal cord of mammals.

Of limbs, the animal has in the thoracic region the maxillipedes which serve the mouth, the great chelae and the walking legs; and in the abdominal or tail region a pair of swimmerets to each segment save the sixth or last, where the limbs are spread out on each side to form flat steering plates.

[1] See an interesting paper(3) by the late Professor of Mental Philosophy and Logic in the University of Cambridge—James Ward in Vol. II of this Journal.

Semisection of the cord in the thoracic region.

This was carried out between the first and second thoracic ganglia and some of the animals made a good recovery. There was loss of tone in the walking limbs, in the abdominal body muscle, and in the sixth abdominal appendage all of the same side as the injury. In about four days the tone of the abdominal muscles returned, the tail ceased to be bent away from the injured side and the animals regained the power of swimming, which they do backwards by powerful strokes of the abdomen. It was noticed, however, that they landed invariably on their backs owing to the lack of steering power due to the continued paralysis of the sixth abdominal appendage of the injured side.

After the shock of the operation was over the swimmerets of both sides resumed their regular beat, but the paralysis of the walking limbs persisted. Therefore the last pair of abdominal appendages which take an important share in the orientation of the animal especially during swimming are so completely controlled by the brain as to be permanently paralysed by semisection of the cord, whilst the other abdominal appendages, the swimmerets, are emancipated from cerebral control perhaps as completely as are the lymph hearts of the frog.

A normal animal placed on its back rights itself by swimming over, the steering being done presumably by the sixth pair of abdominal appendages. An animal with the cord semisected is unable to right itself in this fashion but does so slowly by swinging itself from side to side by alternate pushes on the ground with the great chelæ until the pendulum swing is great enough to topple it over.

The ganglionic decussations are complete enough to permit of voluntary movements, namely walking, carried out very imperfectly, and flapping of the abdomen carried out with vigour and so far as the eye could judge with adequate balance between the muscles of the two sides.

Injury to unipolar nerve cells.

Attempts were made to cut away the nerve cells from beneath a ganglion with a scalpel made by sharpening a needle to a cutting edge. One case was successful. Subsequent sections showed that almost all the cells had been cut away from the under side of the second abdominal ganglion. The immediate disturbance was remarkably slight since stimulation of the cord in the thoracic region was followed by apparently normal movement of the abdominal appendages and abdominal muscles. The object of the experiment was to decide whether destruction of a

unipolar cell affects at once the function of its related nerve fibre. The experiment, so far as it goes, shows what perhaps might have been expected, that there is no immediate effect. The connection between cell and its fibre is solely trophic—if that word has survived into modern physiology.

Relevant papers known to the writer are the following:

1. Yung. Archives de Zoologie, 7. p. 401. 1878.
2. Ward. This Journ. 2. p. 214. 1879–80.
3. Marshall. Studies Biological Laboratories, Owens College, I. 1886.
4. Hardy. Phil. Trans. B, 185. p. 83. 1894.

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SPECIFIC NERVE IMPULSES.

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Introduction.

Gasser and Erlanger(1) have shown that the action potential recorded from an electrically stimulated mixed nerve is "compound" having a series of waves in the falling limb. They have shown by a detailed analysis that this compound action wave is a summation of action potentials in groups of fibres which differ in their physiological characteristics; the most striking differences are those of the rates of conduction.

Gasser and Erlanger found four waves and named them α, β, γ and δ, and they found further that an α wave alone passed to the spinal cord by the motor root, while all four waves, α, β, γ and δ, are found in the sensory root. They suggest that these waves may be concerned with the transmission of specific sensations, and that impulses from different types of sense organs may travel in physiologically distinct nerve fibres. They also bring forward some histological evidence that the different conduction rates are connected with the size of the fibres. But with the cathode ray oscillograph in its present form they are unable to record the "axon action potential" (as they have named the action potential accompanying the activity of a single nerve fibre), and their records are made up of some 1000–100,000 axon action potentials approximately superimposed.

A new form of oscillograph capable of giving a fairly accurate record of the potential changes accompanying the activity of a single nerve fibre has been described in a previous paper(2). The oscillograph was designed primarily to investigate this problem of specific sensory fibres and by its use it has been possible to discover considerable differences in the action potentials accompanying the impulses from different types of sense organ; and further when two types of end organ have been stimulated simultaneously it has been possible to pick out the impulses from each type of organ in a record of the action currents from a common nerve supplying both.

The method.

Preparations from the frog (*Rana temporaria*) were used throughout, partly for simplicity, as the experiments were necessarily somewhat complicated, and partly because Gasser and Erlanger have shown the α, β and γ waves to be very well marked in the frog. The end organs selected were those of skin and the muscle proprioceptors, both skin-nerve and muscle-nerve preparations being readily obtainable.

Preliminary experiments showed that it would be extremely difficult if not impossible to obtain comparable records from two separate preparations. It was seen that all the variable factors which would affect the record and which had been found so hard to control, namely

(*a*) distance between electrodes,
(*b*) degree of drying of nerve,
(*c*) shunting effect of inactive fibres,
(*d*) temperature,
(*e*) tension of nerve,

would be automatically eliminated if the impulses were recorded from a common nerve which branched and supplied both skin and muscle. Impulses could then be recorded from either type of sense organ without disturbing the nerve or the electrodes.

The preparation. A search was made for such a preparation and it was found in the leg of the frog. The lateral branch of the peroneal nerve was dissected out together with a small piece of the skin which its cutaneous branch (n. cut. dors. ped. lat.) supplies. The median peroneal nerve was cut where it rejoins the lateral branch in the foot. All toes except the large one were cut off, a silk thread was attached to the tendons of the extensors of this toe, a pin was forced through the bone near the top muscle attachment, and the bones of the foot severed just above the pin. The preparation was set up as shown in Fig. 1. The common nerve was led into the moist chamber through a slot which was then plugged with cotton wool soaked in Ringer's fluid. The skin was spread out, outer side uppermost, on a piece of moist blotting paper, and was stimulated by lowering on to it a celluloid disc 4 mm. in diameter loaded with 10 grm. The movement of the disc was controlled by a treacle dashpot. The muscle proprioceptors were stimulated by loading a silk thread attached to the toe tendons with a 5-grm. weight controlled by a second treacle dashpot.

The electrodes were short lengths of thread protruding from U tubes full of Ringer's fluid into which dipped silver wires coated with silver

chloride. The exposed parts of the preparation were covered with moist cotton wool which was irrigated from time to time, and this with the

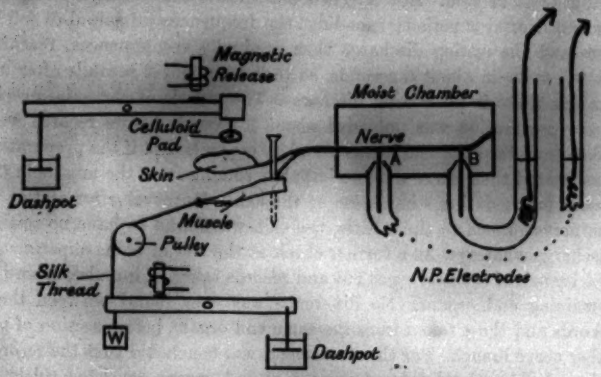

Fig. 1. The skin-nerve-muscle preparation, showing disposition of the electrodes and stimulating arrangements.

moist chamber protected the preparation so well that it has been found alive 6 hours after being set up.

In this preparation it is seen that the cutaneous branch of the nerve is connected only to the skin, and therefore contains fibres subserving cutaneous sensation but no fibres from proprioceptors; whereas the sensory fibres in the muscle branch will come from proprioceptors alone. It was possible to produce at will and to record from the common electrodes *A* and *B* (Fig. 1) action potentials accompanying impulses conveying either proprioceptive or cutaneous sensations. Records could be made of cutaneous and proprioceptive impulses within 5 seconds of each other, and it seems safe to assume that the condition of the nerve will not alter appreciably in so short an interval, so that the records may be taken as comparable in every way.

Precautions. Diphasic recording was usually employed at the beginning of the experiment, and later the nerve was damaged and some monophasic waves recorded.

Adrian(3) has shown that the proprioceptors of the sternocutaneous muscle give a "resting discharge" even when the muscle is unloaded, and the toe muscles employed in my experiments were also found to give rise to a resting discharge of 2–4 impulses per second. This would

normally continue during the recording of skin impulses, and consequently an occasional muscle impulse might turn up in a record of impulses from skin. But Adrian has shown that when a muscle is unloaded after a period of loading, the frequencies of impulses fall to zero and the resting discharge then gradually recommences, reaching its full value in about 5 seconds, so that for about 2 seconds after unloading there is no resting discharge. The muscle of the skin-muscle-nerve preparation was unloaded about 2 seconds before records were taken from the skin, and it was found by controls that if the preparation was treated thus, there were no impulses coming from the muscle in the record taken during stimulation of the skin. Moreover after section of the nerve branch to the muscle, the skin was found to have no resting discharge whatever. As a further check at the end of some experiments one branch of the nerve was cut and records taken of impulses from the remaining end organs. No difference was ever found between these records and those taken from the same end organs before section of the other nerve branch. For these reasons it was concluded that the records taken during stimulation of one set of end organs consisted solely of impulses arising in those end organs and that no impulses from the other parts of the preparation appeared.

Results.

Records of impulses from skin and muscle end organs are shown in Figs. 2 and 3. The records show marked differences[1]. On analysis the potential waves from the muscle are found to differ from those from the skin in the following points:

(*a*) conduction rate,
(*b*) potential gradient of rising phase,
(*c*) maximum potential,
(*d*) time taken to reach the maximum,
(*e*) total duration,
(*f*) potential gradient of falling phase.

The consistency and constancy of these differences is emphasized by Table I in which are given measurements of the constants of some 400 impulses recorded from 11 preparations. Some trouble was experienced with unsteadiness of the base line. This was thought to be

[1] It will be seen that some of the records show a slight initial deflection indicating a positivity at the proximal electrode relative to the other electrode. It is not intended to discuss this here. It was usually associated with the presence of an excess of Ringer's fluid on the nerve.

TABLE I.

	1	2	3	4	5	6	7	8	9	10	11	Mean values
1. Experiment	45 *B*	54 *D*	55 *A*	55 *B*	55 *C*	57 *D*	66 *B*	66 *C*	66 *D*	69 *A*	69 *B*	
2. Date (1928)	18. iii	28. iii	24. iv	25. iv	26. iv	1. v	14. v	15. v	16. v	17. v	18. v	
3. Temperature ° C.	10·5	12·5	13·4	13·4	13·7	15·2	15	14·4	15·0	15·8	15·6	
4. Conduction rate of												
(*a*) cutaneous impulses m./sec.	8·0	10·6	10·4	10·4	10·4	10·8	15	15	14·4	15·8	13·2	
(*b*) proprioceptor impulses m./sec.	11·5	12·6	12·1	13·4	13	13·3	21	21·2	18	21	18	
5. Conduction rate of cutaneous impulses expressed as p.c. of that of the proprioceptor impulses	71	84	85	78	80	81	72	71	77	75	73	77
6. Potential gradient of cutaneous response expressed as p.c. of that of the proprioceptor response	62	63	64	56	64	60	76	63	62	69	63	63
7. Time to max. potential in σ												
(*a*) of cutaneous response	1·8	1·24	—	—	—	—	1·1	—	1·2	—	1·2	
(*b*) of proprioceptor response	1·5	1·12	—	—	—	—	1·0	1·07	1·04	1·0	11	
(*c*) ratio of (*a*) to (*b*) expressed as p.c.	85	89	—	—	—	—	91	89	87	—	91	88·5
8. Total duration in σ												
(*a*) of cutaneous response	5·0	4·3	—	—	—	—	3	3·1	3	2·7		
(*b*) of proprioceptor response	4·3	3·65	—	—	—	—	3 (?)	3 (?)	3 (?)	—	3	
(*c*) ratio of (*a*) to (*b*) expressed as p.c.	85	85										
9. Ratio max. potential of cutaneous response expressed as p.c. of proprioceptor response	80	87	—	—	—	—	78	75	80	74 (?)	81	80

due to irregular dying of the nerve. The 11 experiments in Table I were those having the most steady base line picked out from some 25 experiments. The figures in Table I were determined from the records as follows:

(*a*) Conduction rates were determined from the diphasic records by two methods:

(1) Finding how long after the start the diphasic record begins to differ from the monophasic record obtained subsequently.

(2) Subtracting the total duration of the monophasic response from that of the diphasic response.

Either of these methods gives the time taken for the impulse to travel along the nerve between the electrodes, and if this distance is measured the conduction rate can be calculated. Gasser and Erlanger have shown that the electric response is recorded at the edge of the first electrode proximal to the second electrode. The electrode distance was therefore measured between the near edges of the thread electrodes. But neither of these methods is capable of a high degree of accuracy, as a great deal depends on the clearness of the record and the steadiness of the base line. The second method is probably less accurate than the first, as it is extremely difficult to determine at which point either mono- or diphasic deflections return to the base line. However, the errors are practically the same in the determination for both cutaneous and muscle impulses and the ratio of the conduction rates should be fairly accurate. In Table I no value differs from the mean by more than 8 p.c.

(*b*) Potential gradients of rising and falling phases were determined by measuring the angle with the base line made by a tangent in the region of steepest slope.

(*c*) The maximum potential was deduced from the maximum height of the deflection. The measurements of action potentials from skin and muscle in any one experiment can be compared directly, but different experiments cannot be compared, as the amount of shunting by inactive fibres is not constant. At the conclusion of each experiment a record was taken of the excursion produced by 20 microvolts from a potentiometer, in order to standardize the records.

(*d*) The time to maximum and total duration are measured from the monophasic record, but the former may also be measured from the diphasic record if the electrodes are so far apart that the beginning of the wave has not reached the second electrode until after the crest has passed the first.

The potential gradient. The significance of the differences of the maximum potential and the potential gradient is by no means self-evident. There are two possibilities. It might mean that the potential develops more slowly in the cutaneous nerve fibre and has a lower maximum than that of the muscle proprioceptor nerve fibre; but on the other hand it might also be due to the cutaneous fibres having a higher internal resistance than the proprioceptive fibres, and producing about the same maximum potential. In this case the actual potential gradient in the fibres would be still slightly less in the cutaneous fibre because the time to maximum is longer. If this is so we should expect that the greater the shunting by inactive tissue, the more would the potential at the surface of the nerve depend on the internal resistance of the fibre, and the ratio

$$\frac{\text{potential gradient recorded from muscle fibre}}{\text{potential gradient recorded from skin fibre}}$$

would be expected to alter with different amounts of shunting. In some experiments the shunting by inactive fibres in the tissue had been three times as great as in others (measured by the ratio of the absolute potentials recorded) but no significant change could be detected in the ratio of the potential gradients quoted above. This does not entirely rule out the second possibility but makes it probable that the differences in the recorded potential gradients are chiefly due to differences in the actual potential gradient, and that the internal resistance of the fibres has only a slight influence on them. The possible physiological importance of the potential gradient will be discussed later.

Slower impulses. When the skin was stimulated, and occasionally while the muscle was loaded, disturbances thought to be very slow impulses and resembling those previously noted by Adrian(4) were recorded (see Fig. 4 *H*). They were about one-third the amplitude of muscle impulses and were travelling at about one-half of their rate. Monophasic waves thought to correspond with these also appeared in the records (see *X*, Fig. 2 *F*). Their rising potential gradient was about 22 p.c. of that of the muscle impulses. Exact analysis of the records of these impulses has been impossible as their slight slopes become confused if the base line shows the least unsteadiness.

"Double" impulses. Impulses having approximately the same time relations but twice the amplitude of a single impulse have frequently been recorded (see Fig. 4). If these are due to two fibres being active simultaneously, the two impulses would have to coincide within about

Fig. 2. Specimen records of action potentials accompanying impulses arising in cutaneous and muscular receptors.

Diaphasic records	*A.* Proprioceptor impulses	From the same preparation $T = 14{\cdot}8°$ C.
	B. Cutaneous impulses	Electrodes 18 mm. apart.
	C. Proprioceptor impulses	From the same preparation $T = 15°$ C.
	D. Cutaneous impulses	Electrodes 18 mm. apart.
Monophasic records	*E.* Proprioceptor impulses	From the same preparation $T = 15°$ C.
	F. Cutaneous impulses	
	G. Proprioceptor impulses	From the same preparation $T = 10{\cdot}5°$ C.
	H. Cutaneous impulses	

Time marker for *A*–*F* at top 1 D.V. = 21 σ.

Line = ·01 sec. marked on each record.

N.B. All records read right to left.

0·00025 sec., as if there were a longer interval between them the composite nature of the curve could be detected in the record.

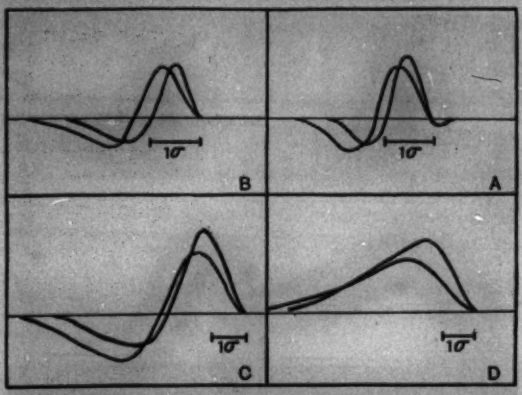

Fig. 3. *A–D* superimposed tracings from enlargements of cutaneous and proprioceptor responses. In each case the slower curve is that of the electric response accompanying the cutaneous impulses.

A. Diphasic, $T = 14{\cdot}8°$ C., electrodes 18 mm. apart.
B. Diphasic, $T = 15°$ C., electrodes 10 mm. apart.
C. Diphasic, $T = 10{\cdot}5°$ C., electrodes 12·5 mm. apart.
D. Monophasic, $T = 10{\cdot}5°$ C.

In one series of experiments 18 such "double" impulses were found among 160 single impulses which followed each other at a mean interval of 17 sigma. The "double" impulses occurred very many times too often to be ascribed to an accidental coincidence.

They might be accounted for in one of the following ways:

A. When a number of end organs are stimulated simultaneously, there may be a tendency for those organs to set up impulses simultaneously.

B. Some fibres may produce a much larger potential at the electrodes than others. Fibres might vary in this respect through one or more of the following causes:

(1) The position of the fibre in the nerve relative to the electrodes.
(2) The internal resistance of the fibre.
(3) The absolute potential developed by the fibre.

Of these B (1) seems improbable, as the proprioceptor impulses from any one preparation are identical within the limits of experimental

Fig. 4. Typical "double" diphasic records shown with single impulses for comparison. L = Distance between electrodes. The line in each record represents ·01 sec.

Records taken during stimulation of muscle proprioceptors:

A. $T = 15{\cdot}0°$ C., $L = 18$ mm. *B.* $T = 15{\cdot}1°$ C., $L = 13$ mm. *C.* $T = 12{\cdot}5°$ C., $L = 14$ mm.

Records taken during stimulation of skin:

D. $T = 15°$ C., $L = 18$ mm. *E.* $T = 15{\cdot}1°$ C., $L = 13$ mm. *F.* $T = 12{\cdot}5°$ C., $L = 12$ mm.

G. Record showing close agreement between responses recorded during stimulation of muscle proprioceptors, $T = 12{\cdot}5°$ C., $L = 16$ mm.

H. Very slow response shown together with a typical proprioceptor response from the same experiment.

error (see Fig. 4 *E*), and there is much evidence that such impulses are not travelling in the same fibre. We must therefore conclude that in these experiments the position relative to the electrodes of an active

fibre in the nerve trunk has no effect on the potential which that fibre produces at the electrodes.

The two latter alternatives, B (2) and (3), are not very probable. The first suggestion, A, however, does not seem unlikely. Adrian and Matthews(5) have shown that the sensory paths from the retina are interconnected, and to a very much less extent the same might be true of the paths from groups of end organs in muscle and skin, and might lead to an occasional synchronous discharge by two or more end organs. Experiments still in progress have lent additional support to this view. This interconnection between end organs might perhaps be effected by nerve networks analogous to those which Lewis(6) has shown to connect the pilomotor muscles of the skin.

Sorting out impulses. It has been shown that the electric response and the rate of conduction of impulses arising from cutaneous and muscular end organs differ very considerably. These differences might be used, within fairly narrow limits, as a method of identifying the origin of sensory impulses.

The records in Fig. 5 show that this is quite a simple matter. These records are comparable because they are all taken from the same

Fig. 5. Records to show the possibility of "sorting" impulses. All were taken in one experiment under identical conditions. $T = 10{\cdot}5°$ C., electrodes 13·5 mm. apart. Line in *C* represents ·001 sec. for all.

A. Typical record taken during stimulation of skin.
B. " " " muscle proprioceptors.
C. " " " both sets of receptors.
D. " " " "

It will be seen that *X*, *X* are of the same type as *A* and so are considered to indicate the passage of impulses arising in the skin, while *Y*, *Y* are the same type as *B* and indicate proprioceptor impulses.

experiment. *A* and *B* show typical impulses recorded during stimulation of the cutaneous and proprioceptive end organs respectively, and below them are shown portions of records taken during simultaneous stimulation of both sorts of end organ. It is at once evident that *X*, *X* are both of the same type as *A*, and *Y*, *Y* are both of the same type as *B*, for this is shown by the initial potential gradients and by the rates of conduction. This is taken to mean that *X*, *X* represent the passage of two proprioceptive impulses and *Y*, *Y* the passage of two cutaneous impulses. In the same way it has been found possible to sort out the impulses in any record taken during simultaneous stimulation of both muscle and skin, and thus to locate the origin of the two sorts of impulses.

Some experiments were carried out to see if it was possible to detect any difference between the impulses set up by driving a fine pin through the skin and those set up by light contact of a 4 mm. celluloid disc, as one might expect these stimuli to cause sensations of pain and touch respectively. But no difference could be detected in the records, and it was concluded that if the constants of the action currents accompanying the impulses produced by pin prick and pressure differ at all, the differences cannot exceed 10 p.c. No impulses were set up in the skin by bringing a hot body near it unless the heat was sufficient to scorch the skin. These results might well be explained on the assumption that cutaneous sensations in the frog are not differentiated.

The response produced by electrical stimulation.

The response of the nerves in the skin-nerve-muscle preparation to electrical stimulation was investigated in order to determine the relationship of the proprioceptor and cutaneous impulses to the α, β, γ processes described by Gasser and Erlanger(1).

Method. The whole of the sciatic nerve was dissected out together with its peroneal branch and the two branches to the skin and toe muscles which were used in the previous experiments. All other branches were cut. The preparation was arranged on electrodes as in Fig. 6. The rotating mirror camera described in a previous paper(2) was used to record the deflections, and the stimuli delivered to the nerve were synchronized with the mirror as before by means of a cam and contact breaker. In this way stimuli could be delivered to either branch of the nerve and the action potentials recorded high up in the sciatic after the impulses had been conducted some distance. The strength of the stimulus was controlled by the rheostats R_1, R_2 (Fig. 6), and made just

maximal for both branches. This was easily effected in the following way. The deflection was observed on the ground glass screen, and the

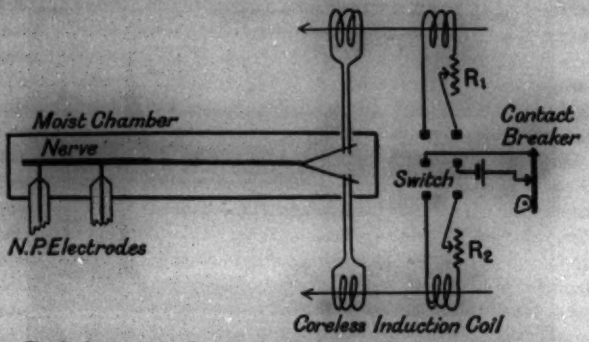

Fig. 6. Arrangement of preparation on electrodes for electrical stimulation.

stimulus gradually increased. As this was done the action potential waves increased rapidly at first, but later remained constant during a considerable further increase of the stimulus. This response was considered maximal, as a still further increase of the stimulus caused the deflection to move forward slightly and then to increase in size, showing that the stimulus was spreading up the nerve and exciting above the fork.

Records were then taken of the action potentials evoked by stimulation of each branch and the lengths of nerve between the stimulating and recording electrodes measured. Typical records are shown in Fig. 7. Table II contains an analysis of a number of such experiments. In

TABLE II.

	1	2	3	4	5	
1. Temperature ° C.	14	15	14·6	14	14·4	
2. Experiment	71	73	73 *A*	74	75	
3. Date (1928)	5. vii	12. vii.	12. vii	17. vii	18. vii	
4. Mean rate of conduction						
(a) of α wave	14·3	17·8	17·5	17·1	21·8	
(b) of pre-α wave	14·5	21·1	19·7	20·1	23·7	
(c) of β wave	10·0	14·9	14·2	14·2	15·9	
(d) of γ wave	?	None	None	?	12·6	Mean
5. Conduction rate of β wave expressed as p.c. of that of α wave	70	83	81	83	73	78

Ratio—musc. impulses : skin impulses. Means 77 p.c. from Table III.

these experiments the motor fibres will have been active as well as the sensory fibres, and the responses of both will appear in the record.

The muscle branch. The action potential wave from the branch of the nerve supplying the muscle is a simple wave. Its rate of propagation,

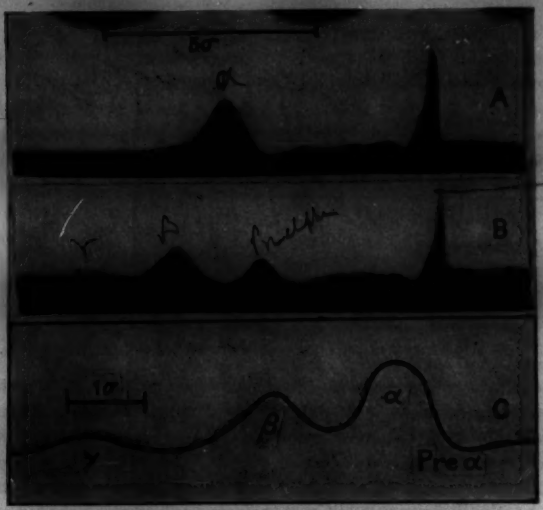

Fig. 7. The monophasic electric responses evoked by stimulating the nerve branches. $T = 15°$ C. Conducted distance 89 mm.

A. Muscle branch stimulated.

B. Cutaneous branch stimulated.

C. Curve obtained by summating curves *A* and *B* to show relative positions of the waves.

Note. The escape of stimulus gives a very large deflection because nearly full amplification was necessary to obtain these records, as they were recorded from the main trunk of the sciatic, and only a small number of fibres was active.

as determined from the time elapsing between the escape of stimulus and the start of the action current, agrees very well with the values of conduction rate of the proprioceptive impulses in the last five experiments of Table I. The disagreement with the earlier experiments may be put down to seasonal variation, as these were performed in the early spring while the electrical stimulation experiments were done in July. Forbes(7) has described alteration in the action current which he considered due to seasonal physiological changes.

As the wave is simple, the motor and the sensory impulses must both

be travelling at the same rate. It is therefore probable that this wave is made up of the α motor and α sensory processes described by Gasser and Erlanger(8). Proprioceptive impulses must therefore be travelling in some of the fibres which give rise to Gasser and Erlanger's α sensory process.

The skin branch. The wave evoked by stimulation of the nerve branch supplying the skin was always double crested. That this double crested wave was not due to distortion by any of the factors which Gasser and Erlanger(9) have shown to yield artificial notches in the record of action potential is evident from the following considerations. First, the impulses arising in the muscle branch have to travel up the same stretch of nerve trunk as those from the skin branch, and would therefore suffer similar distortions. But the former never give rise to anything but a smooth simple wave. Secondly, it was found that the crests gradually separated as the disturbances were propagated up the nerve, and with some preparations from large frogs, where the distance between the stimulating and recording electrodes was about 9 cm., complete separation took place (Fig. 7 *B*).

The conclusion is that the double wave is due to groups of fibres conducting at different rates. It seems, by comparison of the rates of the skin impulses with those of the alpha fibres of the muscle branch, that these two waves can be assigned to the fibre groups which Gasser and Erlanger have called the pre-alpha and the beta groups respectively. The rate of conduction of the beta process agrees very well with the values obtained for the conduction rates of cutaneous impulses recorded in comparable experiments (cf. Table II, 3, 4; Table I, 7, 8, 9 and 10). From these results it appears that the beta process is travelling in the fibres which convey cutaneous sensations.

In some experiments the difference between the pre-alpha and the alpha conduction rates was insignificant, but in others it was considerable and far outside the limits of experimental error. The large pre-alpha wave[1] was most unexpected, for though occasional fast impulses had been detected in the nerve during stimulation of the skin, the size of the pre-alpha wave suggests that nearly as many fibres are conducting at this rate as at the beta rate. What can be the function of these fibres? They might be myelinated motor fibres, but Gasser and Erlanger have shown that the differences in conduction rates of the fibres that enter the cord by the motor root are very slight, and this

[1] In Fig. 7 *B* the two waves have nearly the same amplitude, but more frequently the first wave had an amplitude of half that of the second.

makes it improbable that the pre-alpha fibres are motor. At present their function remains a mystery.

A slow gamma wave was also sometimes present, and its rate corresponds with that of the slow sensory impulses from muscle and skin. In Fig. 7 *C* the curves of 7 *A* and *B* are summated to show the relative positions of the waves. Records very similar to this curve were obtained by stimulating both branches of the nerve at the crotch. It will be seen that there is a striking resemblance between this curve and the compound waves obtained by Gasser and Erlanger when their records are replotted in linear coordinates.

Precautions. It was thought that a single active fibre among a number of inactive fibres might conduct at a different rate from the same fibre surrounded by active fibres. The conduction rates for natural stimulation were obtained under the former conditions, while those for electrical stimulation were recorded under the latter. It therefore seemed worth while to investigate this point. A method was devised which eliminated any distortions of the recording system, so that small differences of conduction rate could be detected.

The procedure was as follows. The skin-nerve-muscle preparation was set up and some diphasic impulses, produced by stretching the muscle, were recorded with the standing wave camera. The nerve was then cut close to the muscle and placed over stimulating electrodes without in any way disturbing the nerve at the recording electrodes. Induction shocks were delivered from a coreless coil, the primary circuit of which was broken by a vibrating contact breaker 30–40 times per

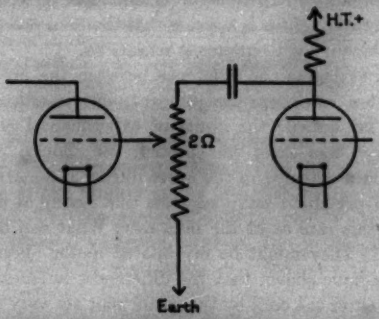

Fig. 8. Method of connecting potentiometer to reduce amplification without seriously disturbing the characteristics of the system.

second. The excursions produced were viewed on the ground glass screen of the mirror camera, and the amplification was progressively reduced until the excursions were the same height as those produced immediately before by single proprioceptive impulses. The excursion was then photographed. The reduction of amplification was effected with a high resistance potentiometer connected between valves 3 and 4, as shown in Fig. 8. In this way the amplification was reduced without affecting the time constants of the amplifier.

The two sets of records thus obtained were found to be identical and any distortions due to the recording system must have been the same in both cases. This result shows that the changes occurring at the electrodes in the two cases had identical time relations. From this result it was concluded that if any difference exists between the conduction rates of the isolated and accompanied impulse, the difference must be less than 5 p.c.

Histology. The nerves of the nerve-skin-muscle preparation were stained with osmic acid, and the size of the fibres measured in camera lucida drawings. The results are expressed in Table III.

TABLE III.

Diam. of fibre	Muscle nerve p.c.	Cutaneous nerve p.c.
Over 10μ	10	12
8–10μ	27·5	9
6– 8μ	52·5	36
Under 6μ	10	43

Although this method can only give a rough estimate of the fibre size, as the nerve shrinks to some extent during preparation and embedding, it is clear that the muscle nerve contains a larger proportion of big fibres than the cutaneous nerve, while the latter has more very big and more small fibres than the former. The records of Fig. 7[1] and Table II show that the muscle nerve is mostly made up of fibres conducting at about 18 metres per sec., while the cutaneous nerve is largely made up of two groups of fibres conducting at about 21 and 15 metres per sec. respectively. It therefore seems probable that the conduction rate is related to the size of the fibres. Gasser and Erlanger(10) have previously suggested that this may be so, and Langley(11) has found a larger proportion of big fibres in muscle nerves than in cutaneous nerves.

[1] In Fig. 7 *B* the two waves have nearly the same amplitude, but in most experiments the first wave was much lower than the second. This agrees well with the relative proportions of large and small fibres found in the cutaneous nerve.

Discussion.

All the results described in this paper indicate the existence of some connection between the conduction rate of a fibre and the type of end organ it supplies. Further, the results confirm Gasser and Erlanger's work in showing some relation between conduction rate and size of nerve fibre. It therefore seems probable that a fundamental connection may exist between the nature of the end organ and the size of the fibre that supplies it. Langley's histological observations(11) on various nerves led him to suspect that this might be the case, and that the connection might have an evolutionary basis.

Erlanger and Gasser have discussed the possibility that the specific form of the impulses set up in the fibres from different end organs may account for the specific effect of the sensory message—the quality of sensation, and the nature of the reflexes it produces. Evidently there are three ways in which a nerve trunk could transmit a representation of the quality of the stimulus to an end organ. It might be carried by:

(*a*) the allocation of the fibres in which impulses are travelling,
(*b*) the nature of the impulses,
(*c*) the grouping of the impulses.

Possibly all of these characters may help to constitute the quality of the message arriving at the cord and the effects of its arrival.

At present we have no evidence of specific grouping of impulses, but the experiments described in this paper have shown that impulses from a stimulus of one quality differ physically from those set up by a stimulus of another quality. Thus the quality of the stimulus may be transmitted in part by the peculiar nature of the impulses set up. For it seems that a specific receptor connects with a specific size of fibre having specific physiological characteristics. It is well known that the physiological properties of motor fibres seem to be connected with the type of tissue in which they end. For example, Lapicque and Legendre(12) have shown that the fibres supplying red muscle differ in chronaxie from those supplying white muscle. Further, Lapicque and Legendre showed that nerves having the shortest chronaxie have the largest diameter, and perhaps the fundamental difference in the motor fibres is also one of size, and this may determine the physiological characteristics, as it seems to do in sensory fibres.

It seems not unlikely that physical differences in the impulses set up by different stimuli may play a part in determining the effect they

produce on the central nervous system, both as regards their transmission to the brain and also the reflex effects they evoke. The specific nature of afferent impulses was suggested by Head(13) to explain the regrouping of sensory messages which he found to occur on their entrance to the spinal cord. Head further suggested that impulses arriving at the cord were discharged into secondary systems, each guarded by its specific receptor. Gasser and Erlanger(10) have pointed out that if the impulses set up by different types of stimuli differ in their potential gradients, an impulse having a certain potential gradient might be an adequate stimulus for one of these receptors, while an impulse having a different potential gradient would be adequate for another. The adequacy of an impulse as a stimulus to specific secondary receptors might be due to the potential gradient of the electric response itself or to the gradient of the events underlying the electric response. For differences in the electric response from different fibres indicate that the fundamental changes, of which the electric response is perhaps only a by-product, also differ.

It has been shown in this paper that the characteristic form of the impulses in a nerve trunk makes it possible to detect whether they have been produced by tension on a muscle or by pressure on the skin. Whether the fibres from the various types of sense organ in the skin are sufficiently differentiated in size to give impulses characteristic of each type is an open question. In the frog's skin there is no clear difference between the impulses produced by touch and prick, but both may arise from the undifferentiated endings in the epidermis. It is also uncertain whether the characteristic form of the impulse would be preserved in the terminal branches of the sensory fibres within the central nervous system. But if it is preserved the differences in the potential gradients of the impulses from various sense organs may well play an important part in deciding their fate in the central nervous system.

Note. It has been tacitly assumed in this paper that the lag of the recording system was negligible and that the records represented the actual potential changes occurring at the surface of the nerve. This is not strictly permissible, but the order of the distortions involved has been considered, and they are very small. The comparisons are made between curves of the same order of time relations, which therefore must suffer the same order of distortions, and the differences between these curves will represent very closely the differences between the potential changes that caused them. It will be seen from Tables I and II that the values obtained for these differences in a number of experi-

ments agree closely; none differs from the mean by more than 8 p.c. The accuracy of the method in these experiments is limited by the sharpness of the photographic record much more than by errors introduced by the recording instrument.

Moreover the chief interest of this investigation lies in the demonstration of differences between impulses from different types of end organ. Such distortions as the instrument has will tend slightly to reduce these differences, so that if for any reason the accuracy was somewhat less than it is believed to be, this means that the actual differences between action currents are even greater than those recorded.

Appendix. The standing wave camera.

A special "standing wave" camera has been designed and made recently. Its essential features are shown in Fig. 9. It has a rotating

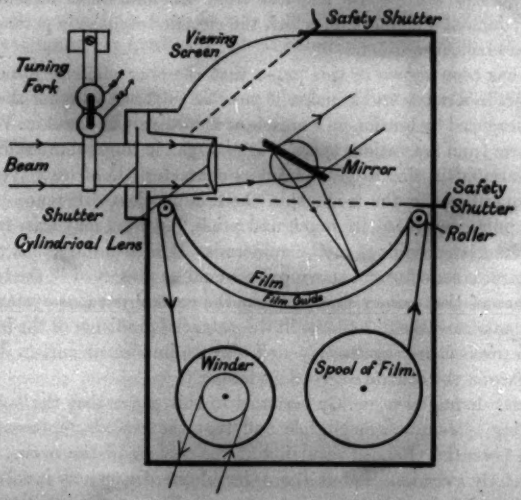

Fig. 9. Diagram showing essential features of the standing wave camera.

mirror driven by a clockwork motor which throws the light on to a strip of Ciné bromide paper bent in an arc about 20 cm. long; records of this length can be obtained with it at an effective speed of 1–3 metres per sec. The shutter is electrically synchronized with the rotation of

the mirror, so that on release of a trigger the shutter opens for only one rotation of the mirror, and the paper is exposed once. The winder can then be turned to bring a fresh length of paper into position. A second motor is sometimes used to drive the winder, and the shutter left open for several revolutions of the mirror. Then owing to the movement of the winder each image is received on a fresh strip of paper. In this way "sample" records can be taken every second at about 2 metres per sec. over a considerable period.

This camera was employed to take most of the records shown in this paper.

Summary.

(1) A method is described whereby the electric responses accompanying single impulses from skin and muscle receptors can be recorded under identical conditions, by leading off from a nerve which branches and a branch supplies each type of receptor.

(2) Considerable differences exist between the electric responses accompanying cutaneous and proprioceptor impulses.

(3) "Double" responses have been found, and are thought to be due to the discharge of two end organs being in some way synchronized.

(4) It is shown that it is possible to sort out impulses coming from muscle and skin by the differences in their electric response.

(5) The response of the nerve branches to electrical stimulation is examined, and reason given for supposing cutaneous impulses in the frog to travel in the β potential wave (described by Gasser and Erlanger) and the proprioceptor impulses in the α wave.

(6) It is shown that the constitution of these nerves lends support to the view that conduction rate and fibre diameter are related.

(7) Specific impulses are discussed. It is suggested that they may play a part in the segregation of impulses into definite tracts in the spinal cord.

I wish to express my gratitude to Prof. Adrian for his valuable criticism and encouragement.

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612.28

THE NERVOUS REGULATION OF RESPIRATION.

Response of the human subject to interruption of the air supply.

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In 1914 Christiansen and Haldane(1) studied the Hering-Breuer reflex(2) in healthy adults by distending their lungs with air at a known pressure. In 1916 Haldane and Mavrogordato(3) attacked the problem of correlating the reflex with the effect of carbon dioxide on the respiratory centre. By closing a tap close to the lips they interrupted the flow of various gas mixtures between a reservoir and the subject's mouth. When ordinary air was used a tracing of pressure changes in the subject's respiratory passages showed on interruption a pressure change of about 4 cm. of water. This pressure was constant for about 3 sec., then rose gradually until at the end of 12 or 15 sec. it reached a height of about 35 cm. It was then succeeded by oscillations of pressure. Assuming the oscillations to be caused by thoracic movements, their onset marked the moment when the lungs had reached a position at which the Hering-Breuer reflex could produce a reversal of the phase of respiration(4). In similar tests performed on students, Bazett(5) found that, in 40 p.c. of the subjects, oscillations commenced in the respiratory passages immediately after the interruption. Boothby and Berry(6) repeated the procedure of distending the lungs, but failed to obtain the cessation of thoracic movement which Christiansen and Haldane(1) had described. The present investigation sought to determine whether the immediate onset of pressure oscillations in the respiratory passages was due to inability of the subjects to allow their respiration to proceed without interference, or whether it was due to causes which were not of a voluntary nature.

Methods.

Kymograph tracings. The apparatus used by Haldane and Mavrogordato has already been described(3) and a tracing is reproduced in Haldane's book *Respiration*(7). Both accounts should be consulted.

One tracing was taken from the subject's air passages, and a second was obtained by recording the movements of a bag containing the gas mixtures presumably as described in a paper by Haldane and Lorrain Smith(6). In the present investigation tracings were taken (*a*) from the chest about the level of the fourth rib in front, (*b*) from just below the level of the xyphoid process, and (*c*) from a water manometer which registered changes of pressure in the respiratory passages together with (*d*) a time tracing (Fig. 1). No reservoir of gas mixtures was used as

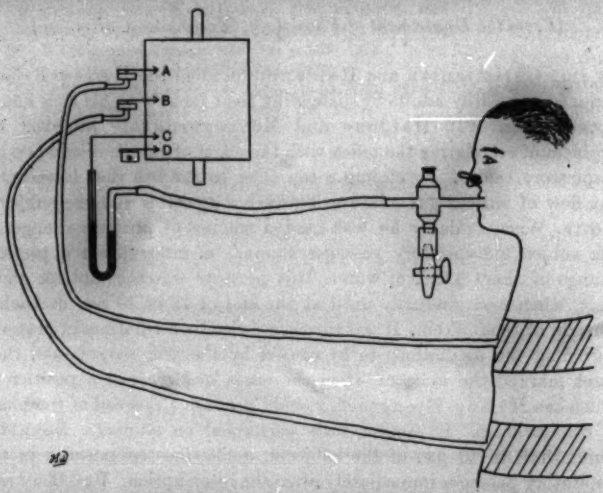

Fig. 1. Diagram to show interruption of air supply. The drum was screened from the subject's view. The distance from the cylindrical part of the mouthpiece to the lips was much shorter than in the drawing. The tube from the mouthpiece to the manometer had an internal diameter of 2 mm. The mouthpiece contained no valves and was closed above. The tracings on the drum are from (*A*) the upper thorax, (*B*) the lower thorax and abdomen, (*C*) the pressure in the respiratory passages, (*D*) the time marker.

only ordinary air was to be breathed. A tap was attached to a Douglas mouthpiece by a short piece of wide rubber tubing through which the subject both inspired and expired, his nostrils being closed by a clip. The dead space of the apparatus was at first 123 c.c. and in the last few experiments 100 c.c.

Radiographic tracings. These were made by means of a grease pencil on a fluorescent screen. They showed the range of diaphragmatic or other movements and were transferred to tracing paper for permanent records.

Subjects.

Haldane and Mavrogordato worked on subjects who were used to investigation of their respiratory functions. Bazett(5) studied students. In the present case the subjects belong to two classes: students who had no previous experience of respiratory work beyond using a mouthpiece and Douglas bag to collect expired air, and healthy adults with experience of such tests. The students sat comfortably in an arm chair in a quiet laboratory and were provided with reading matter. In order to eliminate as far as possible any element of surprise or anticipation they were told in almost identical words that a respiratory tracing was wanted and would be explained later: that they were to be ignorant of what was expected: that the tap would be turned off for a few seconds three or four times but not for long enough to allow carbon dioxide to collect in their blood. False movements, as though to turn the tap, were usually made in order to see if the subject's attention was engaged by reading or if any alteration in his respiratory movement would be produced through expectation of the procedure.

The five subjects of the second class knew the problem under consideration but concentrated their attention, as far as possible, on the reading matter provided.

Results.

(1) *Kymograph tracings.* (*a*) *Untrained subjects.* The pressure alterations in the respiratory passages were recorded in 136 tests on 29 untrained subjects, each subject being tested four or more times in different phases of the respiratory cycle. In only seven tests, that is to say, in only 5 p.c. of cases, did a gradual change of pressure occur which could be interpreted as a prolonged respiratory phase. In the other 129 tests on untrained subjects, interruption of the air supply was followed immediately by oscillations of pressure (Fig. 2). It has been usual to conclude from oscillations on the tracing made from the respiratory passages that simultaneous movements of the thorax are taking place, but this was not found to be so in all cases. In 124 tests, where tracings were obtained from the upper and lower parts of the chest as well as from the respiratory passages, cessation of thoracic and abdominal movement occurred 34 times, that is to say, in 27 p.c. of cases instead

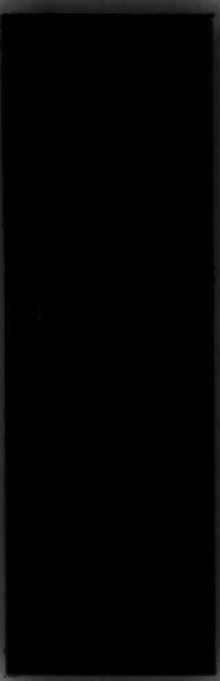

Fig. 2.

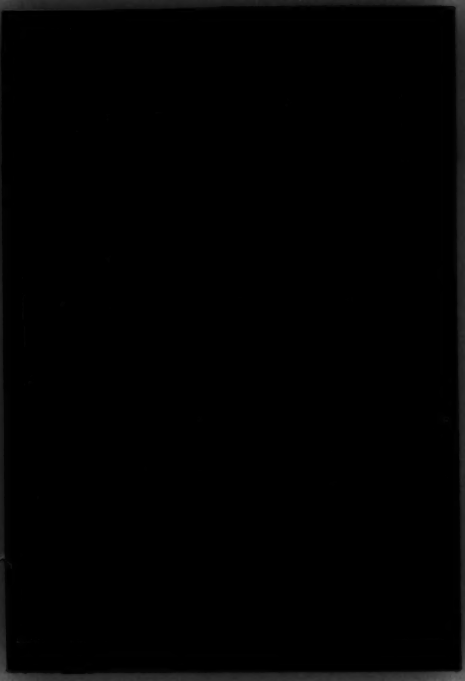

Fig. 3.

Fig. 2. Tracing from an untrained subject. Tracings from top to bottom are (1) thorax, (2) abdomen, (3) pressure in air passages, (4) time in seconds. Inspiration on all thoracic and abdominal tracings is upwards. Inspiration on all pressure tracings is downwards. Positive pressure on pressure tracings is upwards. Actual pressure of water = four times height shown in figure. Arrows marked *C* = closure of tap. Arrows marked *R* = opening of tap. No period of quiescence after interruption.

Fig. 3. Effect of voluntary inhibition of cheek movement. Tracings and arrows as in Fig. 2; *a* = alignment mark. At the first interruption no precautions were taken and slight chest movements can be seen on the thoracic and abdominal tracings. Oscillations of pressure in the respiratory passages are obvious. At the second interruption cheek movement was voluntarily inhibited and the "pressure tracing" approximates to that given by Haldane, while the chest and abdominal movements are less definite. The smallest oscillations on the tracings are due to heart beats.

of in the 5 p.c. based on the occurrence of a gradual change of pressure in the respiratory passages.

(*b*) *Trained subjects.* The test was carried out on five trained subjects. Four of these have been doing respiratory work frequently, the fifth has done a large amount formerly but none during the past two years. None of the subjects gave a smooth line in the tracing from the respiratory passages, but Subject 4 can do so if he voluntarily inhibits cheek movements (Fig. 3). Movements of the neck muscles were seen in Subject 5 as soon as the passage of air was interrupted. Inhibition of his cheek movements did not produce a pause in thoracic movements and the neck movements could not be inhibited. Excluding the tests on this subject, the thoracic and abdominal movements ceased on 12 out of 18 occasions, or in 66 p.c. of cases.

(2) *Radiographic tracings.* A radiographic examination was made on four subjects whose thoracic and abdominal movements ceased but in whose respiratory passages an oscillation of pressure began as soon as the interruption of air occurred. It was then seen that the cessation of thoracic movement lasted for periods of between 3 and 13½ sec. from the time of interruption, while the oscillations of pressure, as explained below, were due to extra-thoracic events.

(3) *Extra-thoracic movements.* This observation suggested that the oscillations of pressure found on the tracing from the respiratory passages were due not to thoracic movements which the stethograph system failed to record but to extra-thoracic movements. The tests were repeated both in daylight and with the radiographic screen. Slight movements, of which the subjects were unconscious, were found in the hyoid group of muscles, in the alæ nasi proximal to the nose clip and in the cheeks. That such movements are sufficient to cause pressure changes similar to those seen on the earlier tracings is proved by the tracings shown in Figs. 3 and 4. Fig. 3 shows a tracing made by a subject when he paid no attention to his cheek movements and when he voluntarily inhibited them. It will be seen that inhibition of cheek movements not only prevented oscillations of pressure in the respiratory passages but also abolished abdominal respiratory movements during the first 15 sec. after the interruption. Fig. 4 shows alterations of pressure due to voluntary movements of the alæ nasi and of hyoid muscles, or to voluntary extension and flexion of the head. In one case, where no extra-thoracic movements could be definitely established by inspection, a radiographic screen examination showed a vertical movement of the cartilage and structures attached to it (Fig. 5).

Fig. 4. Effect of extra-thoracic movements on the "pressure tracing." Tracings and arrows as before. Interruption 1 shows the effect of voluntary inhibition of cheek movements. No. 2 shows the effect of voluntary movements of the alæ nasi proximal to the nose clip. No. 3, shows the effect of voluntary movement of the pharynx in the thyroid region. (These last two movements cannot be dissociated voluntarily.) No. 4 shows the effect of slow nodding movements of the head with precautions against pulling on the apparatus.

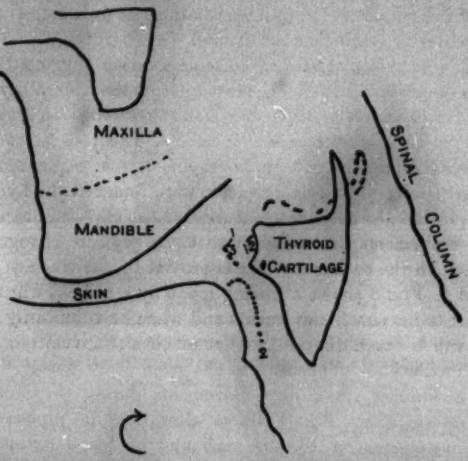

Fig. 5. Tracing from fluorescent screen to show movements of thyroid cartilage. Head in lateral position. The plain line shows the position of the thyroid when the air supply was free. The dotted lines numbered 2 and 3 show other positions reached by the thyroid shadow in its somewhat rotary movement after the air supply had been interrupted.

Discussion.

Extra-thoracic movements, then, will explain most of the discrepancies between tracings from the chest movements and from the respiratory passages. The former give a truer representation of the thoracic movements, but the latter are necessary in order to study, in conjunction with radiographic methods, whether extra-thoracic movements occur. It is important to recollect that movements of the alæ nasi are not necessarily accompanied by movements of the thorax.

The period of quiescence which sometimes occurred after interruption and preceded movements of the thorax must be considered. Haldane and Mavrogordato found it in all cases.

Fig. 6. Interruptions with a pause in the pressure tracing obtained from an untrained student. Tracings and arrows as in Fig. 2.

Bazett found it in 60 p.c. of cases. In the present series it occurred in 27 p.c. of cases among untrained subjects and in 66 p.c. of cases where trained subjects were investigated. The constancy of its appearance in Haldane's subjects suggested that, when the chest and respiratory

passages are made a closed space, the lungs may take longer to reach a position at which the Hering-Breuer reflex can produce a reversal of phase(4). In that case a period without oscillations of pressure would be normal, and its absence might be due to the subject unconsciously forcing on his breathing when interruption occurred.

On the other hand, it is certain that a given subject may fail to exhibit a regularity of response on different occasions. One subject, after showing at first a period without oscillations, realized that he had held his breath and afterwards showed no such delay before oscillations began. Another subject who showed it in four tests when first examined (Fig. 6) failed to do so when the tests were repeated on two subsequent days. It is at least possible that a "delay" in the commencement of pressure oscillations may be due to the subject holding his breath owing to surprise at the sensation produced, to fear that his breathing may be hurried, or to voluntary inhibition of extra-thoracic movements.

Summary.

(1) On interruption of the air supply to the human subject, even if no thoracic movement occurs immediately, oscillations of pressure due to extra-thoracic movements may appear at once on a tracing made from the respiratory passages.

(2) The period of quiescence which sometimes intervenes between interruption and the succeeding oscillations of pressure is often absent.

(3) Subjects who have once shown the period of quiescence do not necessarily do so when the test is repeated.

I wish to thank those colleagues and students who acted as subjects, especially Professor Raper and Dr F. W. Lamb, whose helpful advice has always been at my disposal. Mr P. J. Conlan's willing aid must also be gratefully recognized.

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612.461.61

ON THE RENAL THRESHOLD FOR CHLORIDE IN MAN.

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Introduction.

The conception of a renal threshold for chloride seems to rest more upon analogy and inference than upon precise observation. It is still true, as Haldane and Priestley(6) said in 1915, that "the stimulus to the excretion or the retention of this or that substance by the kidneys is presumably a slight increase or diminution in its concentration in the blood plasma; but quantitative data on this subject are as yet hardly existent." The idea of a "threshold" originated with Claude Bernard(3), who described it in the excretion of glucose. Magnus(7) extended it to chloride: he injected sodium chloride and sodium sulphate solutions into the veins of rabbits and dogs and observed the resulting diuresis and the concentration of the salts in blood and urine; after sodium sulphate injections he obtained urine which was almost chloride-free, secreted by the kidneys from blood which contained 0·6 p.c. of NaCl; so he came to the conclusion (which he unfortunately extended to the sulphate as well as the chloride) that the kidney acts as an "overflow-valve," excreting chloride when the amount in the blood exceeds "a certain threshold," and to all intents and purposes excreting none when it sinks below that threshold.

The idea was taken up by Ambard(1) in his attempt to represent the behaviour of the kidney by algebraical equations. He dealt first with urea, and developed his well-known formula and his "ureo-secretory constant." Turning to chloride, he accepted the idea of a threshold as used by Magnus(7), and gave some data(2) in support of it. These consist of determinations of serum chloride in two human subjects, with corresponding determinations of the rate of excretion of chloride over short periods; the figures show that, with falling serum chloride, the chloride excretion falls at a rate, which, if continued, would bring it to zero when the serum chloride is about 0·565 p.c. Ambard(1) also

observed that in a normal subject living on a low chloride diet, the serum NaCl did not fall below 0·581 p.c. He then made two assumptions: (*a*) that his urea formula is applicable to the excretion of chloride, provided that the total serum concentration in the case of urea be replaced by the excess of serum chloride concentration over a threshold, (*b*) that a "chloro-secretory constant" exists, identical numerically with the "ureo-secretory constant" in a given individual. Making those assumptions, he proceeded to calculate the chloride threshold in various subjects, from determinations of the serum chloride concentration, the urine chloride amount and concentration, and the ureo-secretory constant. He found that the threshold was not fixed, but varied: it rose after a meal, and it also rose slightly when the subject lived on a low chloride diet. Finally he concluded that "the mobility of the threshold for Cl is the essential factor in the regulation of the concentration of Cl in the blood." Ambard's chloride threshold, then, is arbitrarily defined in terms of an equation whose validity and whose constant are assumed, and he finds the threshold to be movable, not fixed.

Cushny(4) uses the idea of threshold to classify urinary constituents into "threshold bodies" and "no-threshold bodies." Chloride is a "high-threshold body," resembling sodium and glucose in "possessing a definite threshold above which it is excreted by the kidney." Cushny crystallizes the idea into a simple formula:

$$\frac{C_B - T}{C_U} = K,$$

where C_B and C_U are the concentrations of chloride in blood and urine, T is the threshold, and K is described vaguely as a "constant for each sample of urine"; but he gives no evidence to suggest that this formula can be applied quantitatively, and in fact he admits that "the relation between the chloride of the plasma and of the urine cannot be followed very exactly."

Observations on chloride excretion in pneumonia are often quoted in illustration of the threshold phenomenon, in spite of the fact that the kidney of a pneumonic patient cannot be regarded as normal. They show that in pneumonia the plasma chloride concentration is unusually low, and the urinary chloride excretion may be very small, but not zero; for instance, the lowest 24-hour excretion of chloride found by Peabody(9) in pneumonic patients was 0·13 grm., while Haden(5) recorded 0·05 p.c. NaCl in the urine of one case, and a "faint trace" in two others.

There is in addition one striking observation recorded by Moss(8) who tested the urine passed by a collier (subject to miners' cramp) after working a shift in a hot damp mine: "it gave not even the slightest cloudiness with silver nitrate, although only 5 c.c. were secreted during 4½ hours."

Speaking generally, then, the facts on which the idea of a chloride threshold is based seem to be:

1. That the plasma chloride concentration is found to vary only within narrow limits, while the urine chloride varies widely in amount and concentration.

2. That as the plasma chloride concentration falls (within those limits), the urine chloride diminishes rapidly.

3. That in pneumonia, where the plasma chloride is very low, the excretion of chloride is slight.

These facts indicate that there is a region of plasma chloride concentration above which chloride excretion by the kidney is relatively great and below which it is relatively small, but they do not justify the statement that a certain fixed level or concentration of plasma chloride exists above which chloride excretion goes on and below which it ceases. The latter is, however, the commonly accepted interpretation of the threshold idea, an interpretation whose precision is not warranted by the available experimental results.

In view of this an attempt was made, in the experiments to be described below, to observe the relationship between plasma chloride concentration and urinary chloride excretion in man, especially at low rates of chloride excretion. Parallel determinations of plasma and urine chlorides were made on two subjects for about a week, during which time they lived under practically uniform physiological conditions, taking a standard chloride-free diet, and accelerating the output of chloride from their bodies by undergoing periods of water drinking and sweating. The latter process is necessary if minimal rates of renal excretion are to be attained within a reasonable time, for when the intake of chloride is reduced to zero the kidneys are tolerably efficient in conserving the chloride already in the body; the skin is much less efficient, and allows the loss of 2 or 3 grm. of sodium chloride in every litre of sweat.

First experiment.

Methods.

The subject, J. G. P., lived for six days on a uniform and completely chloride-free diet consisting of cane sugar, "casein jelly" and water, measured amounts of which were taken at frequent intervals throughout the day. One day's ration of "casein jelly" was made as follows. Forty grm. of "ashless casein" (Glaxo Company), said to contain less than 1 p.c. of calcium, were shaken with a litre of water, and sufficient chloride-free sodium hydroxide was added to make the granules of casein go into opalescent solution (about 7·5 c.c. of 20 p.c. NaOH). The reaction was adjusted by adding 1 p.c. acetic acid till a few drops of added phenolphthalein just gave a faint pink colour. Twenty-five grm. of Coignet's photographic gelatine were added to the solution, and dissolved by warming. Finally 50 grm. of cane sugar and a little vanillin dissolved in alcohol were added, and the mixture poured into 200 c.c. beakers, in which it cooled and set. The resulting jelly was quite palatable. Samples of casein and gelatine had previously been ashed and tested for chloride; none was found. The energy value of this jelly and additional sugar consumed (which varied a little from day to day) was between 1060 and 1580 calories per day.

The experiment occupied eight days in all. The subject's general activities were kept as far as possible uniform; he motored to the laboratory every morning, sat in a chair or moved quietly about the building all day (except when sweating), and motored home in the evening. On the first day he ate a normal breakfast with a generous allowance of salt, and nothing else all day. On the second day he began the diet just described, and returned to a normal one on the evening of the seventh. On each of the first five days he spent two hours (10.30 a.m.–12.30 p.m.) in a closed respiration chamber, in which the atmosphere was laden with moisture, and the wet-bulb temperature raised by an electric heater to between 27° and 30° C. While in the chamber he drank 1500 c.c. of water, and pedalled for four periods of 10 minutes each on a bicycle ergometer, doing as strenuous work as was comfortable in the circumstances. The result was copious sweating on every occasion.

Three times every day the urine secreted in a period of one hour was collected, and a sample of blood was taken from an arm vein exactly in the middle of that period. Urine passed in the intervening periods was also collected. Urines were passed into clean dry measuring cylinders, and measured when cool. Their chlorides were estimated by the usual

Volhard method, duplicate determinations being made on the one-hour specimens. In highly coloured urines containing very little chloride, some difficulty was experienced with the end-point in the titration with thiocyanate. When the nitric acid was added, a brownish colour developed which tended to mask the brown of the iron alum indicator. In these circumstances the titration was done in a wide glass boiling tube held against the light, instead of in the usual white porcelain dish, and a similar tube with its contents incompletely titrated was set up for comparison; in this way satisfactory end-points were obtained. Blood samples were taken from veins at the elbow through a small needle, using only sufficient compression of the arm to make the vein prominent at the moment of puncture. The blood was led directly under paraffin, through a rubber connection and a glass tube which had a weighed amount of heparin (4 mg. per 10 c.c. blood) moistened with saline, on its inner surface. It was centrifuged for 20 minutes and the plasma pipetted off. No trace of hæmolysis occurred, except in one specimen, which was discarded. Plasma chlorides were estimated by the method described by van Slyke(10), in duplicate. In spite of special precautions, including that of stirring during the titration mentioned by Whitehorn(12), the duplicates in a number of cases failed to agree. This was attributed to incomplete digestion of the proteins in the water-baths, which were not self-filling, and had to be re-filled at intervals with cold water. Where duplicates differed by more than 2 p.c. they were discarded; two-thirds of those retained differed by less than 1 p.c. Mean values have been used.

Results.

The effect of the régime on the renal excretion of chloride is shown in Table I, which gives the 24-hour outputs of water and chloride.

TABLE I.

First Experiment.

Of day	Urinary output in 24 hours from 9 a.m. Water c.c.	NaCl grm.	Remarks
1	1995	8·240	Normal breakfast only
2	2326	1·393	Régime begun: chloride-free diet with water-drinking-plus-sweating every morning
3	1398	0·277	
4	2093	0·189	
5	1907	0·122	
6	1411	0·116	(No drinking-plus-sweating)
7	949	0·134	Normal diet resumed at 5 p.m.
8	1199	1·088	

On the first day the chloride excretion was high, because the subject had previously been eating salt freely with his food. From the second day it fell rapidly, and by the fourth day of the régime (fifth day of the experiment) it was as low as 122 mg. in 24 hours. On the sixth day of the experiment it was 116 mg. in 24 hours. On the seventh day, when normal diet was resumed in the evening, fluids were taken freely (800 c.c. in excess of the usual allowance); both salt and water, however, were retained in the body, for the 24-hour urine up to 9 a.m. the following day contained only 0·134 grm. NaCl and less than a litre of water. By the eighth day the NaCl excretion had risen to something over 1 grm.

The chloride concentrations of the samples of plasma, with the corresponding amounts of chloride in the urine, are given in Table II, and plotted one against the other in Fig. 1, where they seem to justify

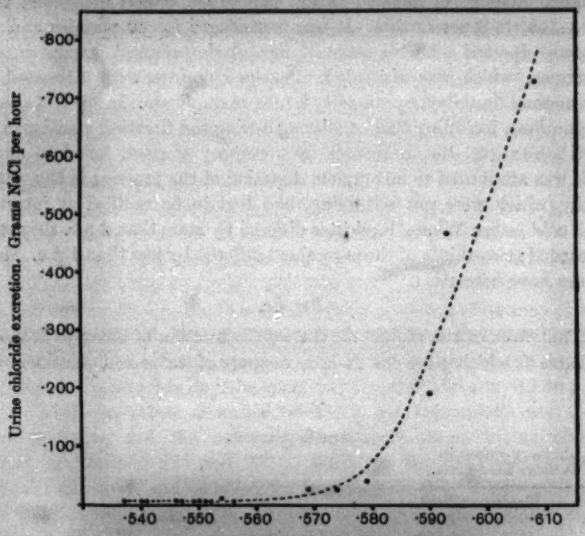

Fig. 1. Plasma chloride concentration and urinary chloride excretion in the first experiment (Table II).

a curve of the shape shown. This curve represents the relation of plasma chloride concentration to urinary chloride excretion in this subject,

under strictly uniform physiological conditions. Its upper part descends steeply; it bends fairly sharply in the region of plasma chloride concentration between 0·555 p.c. and 0·585 p.c., but the number of points obtained there does not fix it very satisfactorily; and below that level it runs horizontally down to 0·537 p.c. This lowest part, which is the most difficult to achieve experimentally, is shown on a larger scale in Fig. 2. The points appear to scatter somewhat, but if those representing

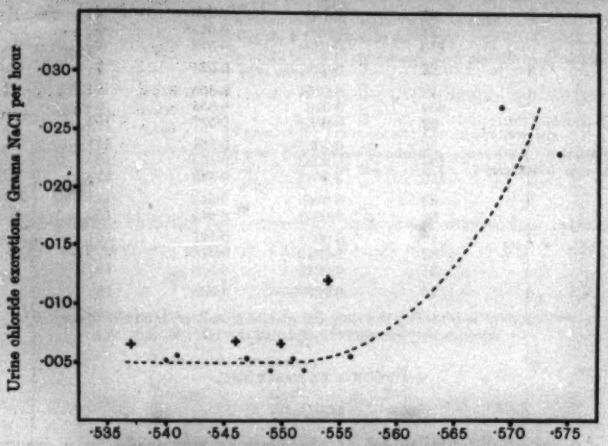

Fig. 2. Plasma chloride concentration and urinary chloride excretion in the first experiment (Table II).

+ Points 9, 10, 12, 13: water excretion over 90 c.c. per hour.
● Points 7, 8, 11, 14–19: water excretion under 60 c.c. per hour.

periods when the water excretion of the kidneys exceeded 90 c.c. per hour be differentiated from the rest (in which it was less than 60 c.c. per hour—*vide* Table II) the former are seen to lie above the curve established by the latter. In other words, at these very low levels of chloride excretion, a diuresis of 100 or 200 c.c. an hour is accompanied by a relatively large increase in chloride excretion.

TABLE II.

Parallel determinations of plasma chloride concentration and urinary chloride excretion at intervals throughout the first experiment.

Day	Urine (1 hour samples) Volume c.c.	NaCl grm.	Plasma NaCl p.c.	No.
1	48	0·727	0·608	1
	29	0·466	0·593	2
	560	0·190	0·590	3
2	63	0·155	0·578	4
	94	0·050	0·511*	5
	150	0·039	0·579	6
3	29	0·023	0·574	7
4	13	0·0054	0·566	8
	259	0·012	0·554	9
	92	0·0064	0·537	10
5	25	0·0052	0·551	11
	121	0·0065	0·550	12
	150	0·0068	0·546	13
6	33	0·0056	0·541	14
	56	0·0051	0·540	15
7	49	0·0053	0·547	16
	32	0·0042	0·552	17
	21	0·0042	0·549	18
8	36	0·027	0·569	19

* This value is evidently in error, and has been omitted from the figures.

SECOND EXPERIMENT.

Methods.

An essentially similar set of observations was made on a second subject, R. S. A., with a special attempt to obtain more points on the transitional part of the curve between 0·555 p.c. and 0·585 p.c. plasma chloride. The sweating periods were a little less severe than in the first experiment. Specimens of blood and urine were obtained twice daily; during the hour while the urine was being secreted and during the preceding half-hour, in each case, the subject remained seated so that circulatory disturbances should affect the kidneys as little as possible. The interval from the last ingestion of food to the collection of specimens was always the same. The diet of casein jelly, sugar and water (1860 cals. per day) was absolutely constant. In other respects the general procedure and technique resembled those in the first experiment, except that plasma chlorides were estimated in triplicate, using improved water-baths, and results agreeing within 1 p.c. were obtained.

The effect of the régime on the renal excretion of chloride and water

is shown in Table III. The reduction in chloride output is rapid, but not so great as in the first experiment.

TABLE III.

Second Experiment.

Urinary output in 24 hours from 8 a.m.			
Of day	Water c.c.	NaCl grm.	Remarks
1	—	—	Normal breakfast Chloride-free diet begun in the afternoon 7·5 grm. NaCl taken in addition
2	2691	6·393	Chloride-free diet continued, with periods of drinking-plus-sweating
3	1957	2·635	" "
4	2057	1·093	" "
5	827	0·499	12 grm. NaCl taken in addition in the evening
6	—	—	13 grm. NaCl taken in addition about 9 a.m. Specimens No. 8, Table IV taken at noon. Normal diet resumed after that

The plasma chloride concentrations and the corresponding rates of urinary excretion are given in Table IV, and plotted in Fig. 3, where

TABLE IV.

Parallel determinations of plasma chloride concentration and urinary chloride excretion at intervals throughout the second experiment.

	Urine (1 hour samples)		Plasma	
Day	Volume c.c.	NaCl grm.	NaCl p.c.	No.
2	142	0·723	0·600	1
	74	0·247	0·586	2
3	55	0·216	0·579	3
4	122	0·112	0·577	4
	65	0·062	0·563	5
5	24	0·034	0·553	6
	163	0·023	0·556	7
6	35	0·183	0·604	8

the scale is the same as in Fig. 1. With the exception of No. 8, the points lie close to the curve shown, which is quite similar to that in Fig. 1, although lying a little further to the left; they establish the transitional bend of the curve where the points in Fig. 1 were too few.

The ingestion of large amounts of NaCl in a short time at the end of the experiment (Table III) led to two interesting results. In the first place the 24-hour specimen of urine completed at 8 a.m. on the sixth day contained only 0·499 grm. NaCl, although 12 grm. had been swallowed the previous evening. This emphasises the degree to which

the body, if depleted of salt, retains what salt may be subsequently offered to it. Along with the salt it retains water, corresponding to that lost during the period of depletion (Veil(11)); hence the low volume, 827 c.c., of the 24-hour urine of the fifth day. Secondly, specimens of blood and urine were obtained about midday on the sixth day, after 25 grm. in all of salt had been taken, and while the restoration of the normal salt and water equilibria of the body was presumably still in process. Their chloride values gave the point 8, which is completely off the curve in Fig. 3, indicating that although the plasma chloride value

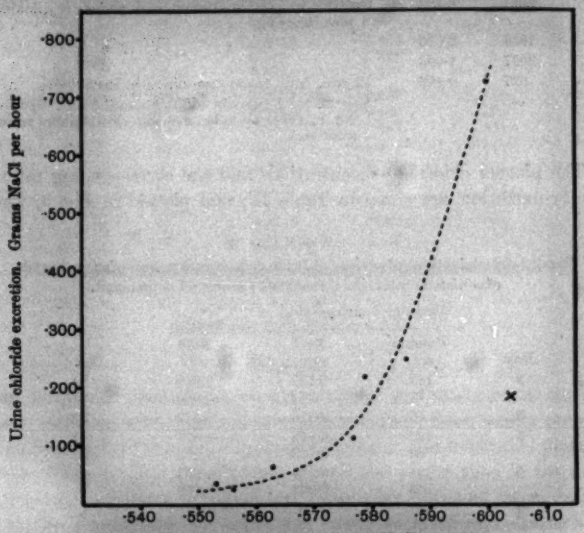

Fig. 3. Plasma chloride concentration and urinary chloride excretion in the second experiment (Table IV).

● Points 1 to 7. X Point 8.

has risen to its initial level the chloride excretion rate is still quite low: the kidney's response to the presence of chloride in that concentration in the plasma has altered.

Discussion.

The conclusion to be drawn from these two experiments is that, in the human subject, if the physiological conditions of diet, water intake, muscular activity etc., are rendered sufficiently uniform, and if the body is gradually depleted of chloride by a chloride-free régime and forced sweating, then the response of the kidneys to variation in the concentration of chloride in the plasma can be defined as a reasonably smooth curve, relating plasma chloride concentration to rate of renal chloride excretion. In the two subjects used the curve is found to be steep above a plasma NaCl concentration of about 0·585 p.c., indicating a large increase in excretion for a small increase in plasma concentration. Below about 0·555 p.c. of plasma NaCl it is horizontal, indicating a steady rate of excretion at about 5 mg. per hour, down at least to 0·537 p.c. of plasma NaCl. The intermediate transitional part of the curve occupies the range 0·555 p.c. to 0·585 p.c. plasma NaCl, and this part of the curve is the most precise definition that can be given of the term "renal threshold for chloride." The threshold is not a definite point, but simply a neighbourhood in which the relation of renal excretion to plasma concentration changes rapidly; below this neighbourhood, the excretion is small and apparently steady, but it is not zero.

There is no evidence that even this roughly defined neighbourhood is constant in physiological (or pathological) conditions other than those of these experiments. On the contrary, the discrepancy of point 8 in Fig. 3 suggests strongly that during such a disturbance as the transition from a low-salt to a high-salt régime, the whole curve will be considerably altered; and the scatter of the points in Fig. 2, already referred to, points to a relationship between rates of excretion of chloride and of water, especially in the lower part of the curve. In other words, the chloride-excreting activity of the kidney is not determined solely by the chloride concentration of the plasma, but is influenced by other factors; therefore it is extremely unlikely that even the transitional neighbourhood, approximately defined in these experiments, is constant in varying circumstances.

These considerations throw doubt on the usefulness of applying the threshold idea to chloride at all. It is obvious that a fixed threshold value on which accurate calculations might be based does not exist. The study of the behaviour of the human kidney would be advanced if the conception of a chloride threshold were discarded, and, under controlled and suitably varied physiological conditions, more extended

observations were made of the kidney's response to the stimulus of varying plasma chloride concentration.

Summary.

1. The relation between the rate of excretion of chloride by the kidney and the concentration of chloride in the plasma has been observed in two human subjects, over a wide range of plasma chloride concentrations, under strictly uniform physiological conditions.

2. The nearest approximation to a renal threshold for chloride appears to be in the neighbourhood of 0·555 p.c. to 0·585 p.c. plasma NaCl, where the above relationship is rapidly altering. There is no evidence that this neighbourhood is constant under varying physiological conditions. It is suggested that the idea of a renal threshold for chloride be abandoned.

I wish to thank Dr J. G. Priestley for his advice and help throughout these experiments, and for his kindness in suffering himself to be the subject of the first one.

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A STUDY OF SOME FACTORS INFLUENCING INTESTINAL MOVEMENTS.

By J. BARCROFT and C. S. ROBINSON.

(*From the Physiological Laboratory, Cambridge.*)

Two methods have been extensively used for studying intestinal movements in live animals, viz. by means of X-rays and by direct observation after opening the abdomen. The first method which has been used in many researches by Cannon(1) has the advantage that the subject is not materially inconvenienced by the process, may live in its usual manner and eat its customary food. The method may also be used on human subjects. It has the disadvantage that the observations are indirect, being, in fact, not observations of the gut itself but of its contents. Neither is it possible by this method to study movements of the empty gut because the presence of an opaque substance in the gut is necessary. By the second method which has been developed chiefly by Alvarez(2) movements of the empty gut may be observed and the observations can be made directly. The application of the procedure is, however, limited by the fact that the subject must be sacrificed, that it is under the influence of an anæsthetic during the experiment and that it has undergone radical surgical treatment in exposing the gut.

A third method which has been described(3) differs from the other two in that the portion of the gut studied is disconnected from the rest of the intestine and hence does not contain the usual food residues nor function in its usual capacity. For experimental purposes it has the advantage that substances can be introduced and expelled at will. But again the observations are indirect.

The method to be described in the present article possesses some of the advantages of each of the others in that it permits the direct observation of the intestine of an animal living its normal life on a normal diet, and that observations can be made of the movements of an intestine which contains no foreign material. Admittedly the intestine is not a normal one but it has undergone a minimum amount of disturbance. The preparation was suggested by a similar one of the spleen(4). It yields a section of intestine (from 2 to 3 inches long on our dogs) lying outside

the abdomen but attached to it, retaining its normal blood supply and, except for its exposure and the effects of its attachment, being a normal organ.

The operation was carried out under chloroform and ether and in all cases but the last was performed in the following way. A transverse incision was made in the abdominal wall 3 to 4 inches in length stretching 1½ to 2 inches on either side of the ventral middle line at about the level of the kidney. The skin was treated as one layer, the rest of the abdominal wall as another. Tissue was cut away from the ends of the incision, making it somewhat dumb-bell shaped. A loop of intestine was withdrawn slightly longer than the incision and so placed that the gut penetrated the wall at the expanded terminal portions of the incision. The loop was then anchored to the body wall by two stitches at each end. These only penetrated the superficial portion of the gut and were removed some days later when adhesion had become secure. Fig. 1 shows the outline of the exteriorized gut 48 and 72 hours after exteriorization.

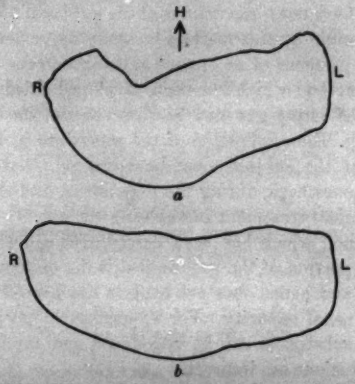

Fig. 1. Outline tracings of exteriorized portion of small intestine. *H* = towards head. *R* dog's right side. *L* dog's left side. (*a*) 48 hours after exteriorization. (*b*) 72 hours after exteriorization. Both show characteristic loss of tone. The centre of Fig. 6 shows approximately the normal diameter of the intestine.

The wall and subsequently the skin were sewn up with sutures passing underneath the gut. No attempt was made to take any precise portion of the small intestine, the part taken being determined post-mortem.

In the last experiment the incision was longitudinal and almost in the middle line. That technique is easier and possibly there is less chance of kinking, but the gut in course of time seemed to get drawn down as a sort of dependent loop into the abdomen assuming the form shown in Fig. 2, which is a section through the intestine 20 weeks after the operation.

Fig. 2. Section through exteriorized intestine, post-mortem after 20 weeks after operation. → The lumen of the gut (not necessarily the centre of the lumen). *A*, portion of intestinal wall drawn down making a loop, portions *C* and *D* having been about 3 inches apart at exteriorization. *B*, body wall. The whole gut was covered inside with villi which were unnaturally short on the exposed surface. Reduced to ½.

The dressings consisted of pads of cotton wool heavily smeared with sterile vaseline placed around the loop. Over it was put vaseline, transparent material and the usual outer dressings.

Some observations were made on four dogs. On two of them systematic studies were carried out over periods of several months and these furnished the material for the present report.

For prolonged systematic watching the dog was placed in a Pavlov stand. At first, observations were made by means of a mirror supported at an angle beneath the exposed section. But it was discovered that such pictures were sometimes indistinct enough to cause inaccuracies in their interpretation. The stand was then raised to a height which allowed the observer, seated in a chair, to watch the section itself. If a more accurate study is to be made some system for mechanically recording the movements might be devised. Perhaps the one used by Alvarez(2) for studies on exposed intestines could be adapted to this purpose. For the present work the section was assumed to be divided into three parts *A*, *B* and *C*, the food entering at *A* and leaving at *C*. Notes were made of the time and place at which the movements started, the type of movement, whether it was "weak" or "strong," and its rate of progress. From the data obtained it was possible to construct a graph showing the activities that took place throughout the course of the experiment.

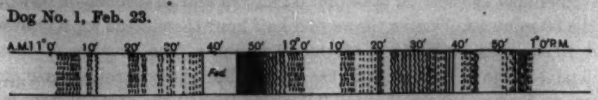

Fig. 3. Effect of feeding on movements in Dog No. 1. Figures represent time. Weak movements shown by broken lines, strong movements by unbroken lines. Static movements by straight lines, progressive movements by wavy lines.

A portion of such a graph is shown in Fig. 3, and the data used in its preparation are given in the sample protocol.

For Figs. 4 and 5 the time before and after feeding was divided into

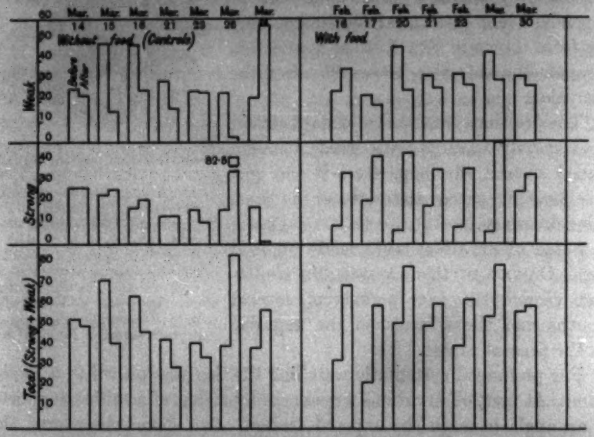

Fig. 4. Observations on Dog No. 1. Ordinate = percentage of half an hour during which the gut was in a state of activity. Each figure consists of two parts, the height only of which is relevant. The first part refers to the half hour before the feeding interval, the second part to the half hour afterwards. During the experiments, March 14–28, no food was given in the feeding interval.

half-hour periods and the number of minutes in each occupied by "weak" or "strong" movements was calculated from the record of the experiment. These were then converted into percentages of 30 minutes and the values so obtained for the pre- and post-feeding periods respectively were averaged to obtain those used in preparing the chart. Thus in the experiment on February 16 (Fig. 4) the dog was observed for an hour before feeding. During the first half-hour period the weak movements of the section lasted for 5 minutes and 35 seconds or 18·6 p.c. of the 30 minutes. During the second half hour they lasted for 9 minutes and 10 seconds or 30·6 p.c. of the 30 minutes. The average of these, 24·6 p.c., is the value shown in the chart. For the control experiments where no food was given (headed "Without food") the same system was used, the dog being merely released from the stand for a few minutes instead of being fed.

This system is manifestly crude and when a quantitative interpretation is attempted is subject to considerable subjective error, but even

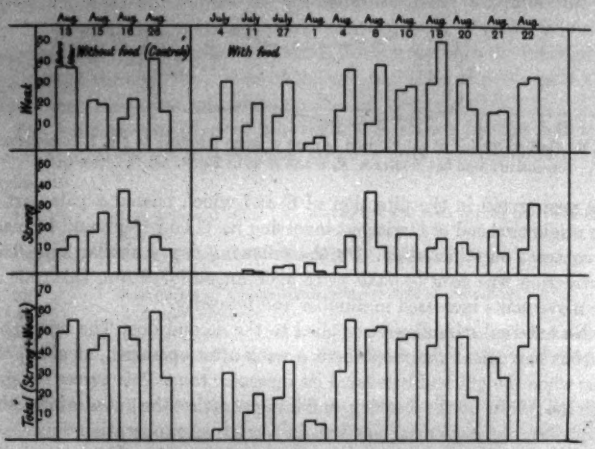

Fig. 5. Observations on Dog No. 2. Plotted as in Fig. 4.

so we regarded a faithful account of naked eye observation as more illuminating than the application of some graphic method presenting the appearance of accuracy but resting on no satisfactory basis. It enabled us, at least, to gain a knowledge of the value of the preparation for the purpose in mind and to add some facts worth recording.

Solid food was withheld from the dog for at least 96 hours after the operation. When given it was consumed more or less completely and the dogs rapidly regained their appetites. From the time of taking food normal stools were passed, so material must have traversed the exposed section.

Time of reappearance of spontaneous movement. Spontaneous movements were not seen for some days after the operation in any dog. The exteriorized portion of the intestine being apparently also without sensation. In the case of the first dog stimuli of various sorts, *e.g.* pinching, electricity and "pituitrin," were applied in an endeavour to induce movement. These efforts were quite unavailing until the fourth day after operation. On this day after manipulation a marked contraction developed which remained stationary for some time. Electrical

stimulation with a strong current for roughly 30 seconds at *A* (Fig. 6) gave a good but local contraction. At *B* it gave a contraction which

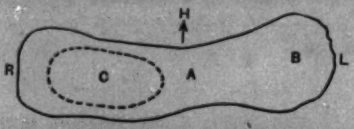

Fig. 6. Outline tracing of exteriorized portion of same intestine as Fig. 1, 96 hours after operation: tone has returned. *R*, *L* and *H* as in Fig. 1. *A*, *B*, *C* (see text).

was propagated in the direction of *C* and which passed a pale part in the neighbourhood of *C* without involving it. *C* however could be made to contract on stimulation. On the following day a similar superficial contraction was seen to take place spontaneously. From this time on the movements increased in number and intensity.

No external stimuli were applied to the second dog. The first spontaneous movement was seen about a week after operation, at about the time when the gut had recovered its muscular tone. This agrees roughly with the results on the first dog, and it appears that the gut was incapable of spontaneous movement until about a week after operation, in spite of the fact that healing was apparently complete and solid matter being forced through the lumen. None of these early movements was progressive; they consisted only of superficial contractions of isolated areas of the intestinal wall.

It is of course possible that movements took place earlier than those recorded, occurring when the dog was not under observation.

Our experience is at variance with that of Babkin and Sinelnikov(3) who found intestinal loops to be much more irritable immediately after operation than later. With our preparation motility was completely inhibited for several days and was recovered very slowly.

This recovery was studied particularly in the case of the second dog. It appeared to be associated with the overgrowth of the epithelium, the active area of the section increasing progressively with this process and being confined exclusively to the area so covered. Movements would proceed to the margin of the epithelium and there stop abruptly. Eventually, however, after several weeks, the remaining unepithelialized portion seemed to acquire the power of motion.

Sequence of recovery. The first movements observed were isolated, superficial contractions which showed no sign of progression and could serve neither to mix nor transport the contents of the intestine. This type of movement was the only one seen during about the first 10 days

following operation. Then the contractions showed a tendency to move down the gut, taking the form of waves or ripples. These movements, infrequent, weak and superficial, gradually increased in number till by the third week they were the predominating type. By the following week they had become sufficiently strong to be a factor in the transportation of food through the lumen of the gut, and their importance in this respect increased from this time on.

The inactive condition of the section is shown by the following record of our experiment performed 11 days after the operation on the second dog.

11° 15′ a.m.	Dog placed in stand. No movement was seen during the subsequent 2 hours
1° 15′ p.m.	Removed dog and fed her on fish
1° 20′ „	Replaced her in stand
1° 33′ „	A suspicion of a contraction in the middle of the section
1° 54′ „	A slight contraction at *A*.
1° 58′ „	Another slight contraction at *A*. No further movement was seen during the period of observation which lasted until 3° 20′, *i.e.* 2 hours after feeding

This series of observations should be compared with those given in the sample protocol as typical of the normal behaviour of a section which has regained its activity. The same thing is shown in Fig. 5 in the increase in duration of movement.

An interesting point to be noticed in connection with these observations is that for several days food was transported through the alimentary canal, and normal stools were passed in spite of the fact that at least 3 inches of the intestine appeared quite inactive. The duration of this inactive period and the fact that the inactivity was restricted to the exposed section indicate that it was not due to the after effect of the anæsthetic used for the operation.

These various relationships are summarized in Table I.

TABLE I.

Dog no.	First solid food given	First stool passed	First spontaneous movement observed	First progressive movement observed
1 (Dowsey)	4th day after operation	4th day after operation	5th day after operation	4th day after operation*
2 (Tivey)	7th day after operation	5th day after operation	One slight movement of unknown character seen about a week after operation	
3 (Dalley)	7th day after operation	8th day after operation	15th day after operation	
4 (Floss)	Not before 5th day	3rd day after operation	One movement of unknown character on the 11th day	

* Induced by strong electric stimulus.

Influence of food. For studying the influence of food on the movements, the dog was not fed for at least 12 hours before starting the experiment. She was placed in the stand and watched, usually for an hour and a half, then removed, fed and returned to the stand, this process requiring, usually, 5 minutes. The observations were commenced at once and continued for an hour and a half or longer.

In all, thirty experiments were made, totalling more than 70 hours' observation on the movements of the exposed intestine of dogs which had had no food for 12 hours or more preceding the experiment. Although the dogs had had no food for even 24 hours the gut was not empty. Frequently the sounds accompanying the movements showed the presence of material in the intestine, presumably secretion which has been shown to be produced more or less constantly even when food is withheld (5).

All types of movements described by other observers have been seen at one time or another in the food-free gut, including the ordinary peristaltic wave, churning movement and segmentation.

The effect of feeding the first dog was to increase both the intensity and duration of these movements. This is shown in Fig. 4 in the increased values for "strong" movements and "total" movement in the "after feeding" section of the chart. These results are not nearly so marked in the case of the second dog as an inspection of Fig. 5 and a comparison of it with Fig. 4 will show. There is not the marked and consistent increase in the "strong" movements shown in Fig. 4, nor is there so pronounced an increase in the total time during which the section was active.

In a general way our observations in this respect agree with those of Sinelnikov. He found a wide variation in the activity of intestinal loops of different dogs and of the same dog on different days.

In some cases the effect of feeding seemed to be an immediate stimulation of motility, while in others the action was apparently delayed for from 10 to 30 minutes. With the assistance of Dr A. E. Barclay, Dog No. 1 was given a barium meal on one occasion and food was seen with the X-rays to pass into the intestine at once. It reached the exposed section about 10 minutes after being eaten. Further evidence on this point was obtained later. The dog swallowed a piece of bone about 2 inches long which lodged in the section and caused the development of a fistula. She was given a capsule of carmine, the contents of which appeared in the drainage from the fistula 10 minutes after the administration of the dye. This interval agrees roughly with that usually required

for the development of marked activity in the section after feeding. The location of the section was found on autopsy to be about 7 feet from the stomach and about 2 feet from the appendix.

Effect of exercise, emotion and sleep. Observations were also made on the effect of exercise, emotion and sleep on the movements. No effect could be seen from any of these on either of the two animals studied. Both frequently slept in the stand. The second one as a matter of routine went to sleep on being reinstated after feeding during which time the movements were very energetic. This agrees with observations by Cannon(1). Both dogs on various occasions were disturbed by the barking of dogs outside the building or by the entrance of visitors without their excitement being reflected in any change in the movements. This is contrary to the observations of Cannon who observed a cessation of movement as a consequence of apprehension. Several experiments were carried out with Dog. No. 1 to determine whether exercise stimulated or inhibited intestinal activity. The results were all negative.

Summary.

A method is described for exteriorizing a section of the intestine for the purpose of studying its movements. Using this preparation observations have been made on the movements of a small intestine of dogs 12 hours or more after feeding, and the effects of various stimuli on these movements as well as on the movements immediately after feeding have been studied.

Even when empty of food the small intestine is fairly active, displaying all the types of movement observed when food is passing through. The amount and intensity of such effects vary with the individual.

Exercise, emotion and sleep caused no noticeable change in the movements.

Food passed down the intestine and fæces were normally evacuated even though a portion of the intestine 2 or 3 inches in length was inactive.

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SAMPLE PROTOCOL.

Dog No. 1, February 23, 1928.

Passive	Active	
10° 59′ 10″–11°	1′ 40″– 7′ 0″	Shallow pulsations and waves
11° 7′ 0″–	9′ 0″–12′ 0″	Very shallow waves becoming moderately strong at 11′ 0″ for a few seconds and then decreasing
12′ 0″–	19′ 0″–21′ 20″	Slow shallow waves
21′ 20″–	21′ 50″–22′ 40″	Very shallow waves
22′ 40″–	26′ 0″–27′ 10″	Shallow waves
27′ 10″–	28′ 0″–29′ 30″	Pulsations in *B* and then waves from *B* to *C*
29′ 30″–	32′ 0″–32′ 30″	Very shallow pulsations
32′ 30″–	33′ 30″–37′ 0″	Very shallow pulsations at *B* with respiration
	The dog was fed on boiled meat	
	45′ 30″–59′ 50″	Strong movements were going on when she was reconfined. Segmentation. The movements were mostly contractile in character. There was strong spastic churning and segmentation with only occasional transportation. At 50′ 30″ only the *B–C* section was active. Then only *A* at 52′ 0″. Then there were waves from *A* to *C*. There was a gradual reduction of energy till 58′ 0″ when only superficial pulsations of *B* and *C* remained
59′ 50″–12°	0′ 20″– 1′ 30″	Shallow, intermittent waves from *A* to *C*
12° 1′ 30″–	11′ 0″–14′ 0″	Shallow intermittent waves from *A* to *C*
14′ 0″–	15′ 20″–39′ 0″	There was a constant succession of very superficial local spasms with intervals of quiet lasting 10–20 seconds. These were almost too slight to record except for an occasional contraction which may have been incited by respiration, the dog being asleep and breathing deeply. At 20′ 0″ there was a period of somewhat greater effort lasting 2 minutes when it increased to moderate intensity for 30 seconds, gradually running down to a faint flickering. There was another increase in activity from 23′ 30″ on. It became moderate till 28′ 0″–29′ 0″ and very strong till 30′ 30″–33′ 0″ with evident transportation and sounds. Then followed a period of shallow contraction and pulsation till 34′ 30″ when these deepened for 10 seconds. The gut seemed to be filling during a period of relative quiet with only contractions and pulsations which deepened from 37′ 30″ to 39′ 0″ though there appeared to be no transportation, and then the gut became quiet
39′ 0″–	41′ 20″–45′ 0″	Slow, intermittent contractions, pulsations and waves interspersed with intervals of quiet lasting 10–20 seconds. The movements became moderately strong from 44′ 0″ to 45′ 0″ though still slow and intermittent. Then there was quiet except for single contractions at long intervals
45′ 0″–	51′ 35″–58′ 0″	Slow waves, contractions, and segmentations becoming strong by 54′ 0″. But little apparent transportation till 55′ 0″ when the movements became strong and more rapid. They began to subside at 57′ 30″ and stopped at 58′ 0″ except for single contractions

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STANDARDIZATION OF THYROID PREPARATIONS.

By J. R. MØRCH.

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I.

In 1919 Kendall succeeded in isolating a chemically pure substance, thyroxine, from the thyroid gland, and this substance appeared to have the physiological effect characteristic of the gland. Later (1925) Harington prepared this compound synthetically. It therefore might seem needless to try to find a method for the standardization of thyroid preparations as they might be expected soon to be replaced by the chemically pure substance. This, however, is not likely to come to pass very soon. For it is not a foregone conclusion that the effect of thyroxine is identical with that of the dry gland; various findings indicate that the gland contains active substances other than thyroxine; and, besides, the preparation of thyroxine, by synthesis as well as from the gland, is such a difficult and expensive process that the price of this substance is much higher than is the price of the desiccated gland.

The simplest method proposed for the standardization of the dry gland consists in simple determination of the iodine content, and a number of pharmacopœias still stipulate a minimal content of iodine, on the assumption that the amount of iodine necessarily implies the corresponding amount of thyroxine which contains about 65 p.c. of iodine. This iodine content, however, is not a reliable measure of the physiological effect of the dried thyroid gland, inasmuch as the iodine content of the preparation can be increased as much as one pleases by the admixture of iodine-protein compounds which are difficult to detect; and also iodine compounds introduced into the organism are stored to a large extent in the thyroid without entering into the specific organic combination; and, finally, it has been found that the thyroid of children often contains no iodine, although there had been no sign of impaired glandular function while the child was alive (Storm van Leeuwen), so that the low iodine content of a preparation does not necessarily indicate that the preparation is worthless.

Thus an analysis of the iodine content of a preparation gives no definite information as to its therapeutic value, and a biological method must therefore be employed for this particular estimation.

As all biological methods are based on the power of the substances concerned to influence certain physiological functions, we have an excellent basis for the elaboration of such a method in the large experimental material illustrating the complex function of the thyroid which has been published in the last decade.

Of the biological reactions which may be thought practicable—and have in part been tried out, the most important for this purpose are the effect in:

(1) acetonitrile poisoning in white mice,

(2) the course of metamorphosis in frog and salamander larvæ, and

(3) the metabolism in warm-blooded animals, determined directly.

1. *The effect of dried thyroid gland on the course of acetonitrile poisoning in white mice.* The susceptibility of certain animals to different poisons changes considerably when thyroid gland is given as well. Thus Hunt(1) has observed that white mice become more resistant, guinea-pigs and rats less resistant to acetonitrile when thyroid tissue is fed to the animals beforehand; the toxicity of morphine, on the other hand, is increased for all the animals mentioned. The observations by Hunt on the effect of thyroid on the toxicity of acetonitrile to white mice form the basis for Hunt and Seidell's(2) method for standardization of thyroid preparations; for a number of preparations they determined the smallest dose which, on daily feeding for a number of days, was able to protect the mice against a dose of acetonitrile that was fatal to normal mice. The results obtained with this method, in its original form, have but little precision, and this has proved to be due to the fact that the course of the poisoning process is influenced strongly by a number of circumstances (sex, age, food, temperature of surroundings, etc.). Nor did Hunt find any proportion between the total amount of thyroid ingested and the increase of resistance to acetonitrile acquired thereby; only in a very general way could it be said that differences existed in the effect of different doses of thyroid. More recently Haffner and Komiyama(3) have modified Hunt's method and described the precautions necessary in order to avoid disturbing influences which made it uncertain. From the experiments reported by Haffner and Komiyama it looks as if their method gives values which have a certain degree of reliability (30 p.c. accuracy). The principle of the method consists in feeding thyroid by tube in varying dosage once only to a series of mice; 24 hours

after the thyroid feeding, acetonitrile is injected in decidedly lethal doses. Haffner and Komiyama determine the smallest dose of gland which can protect a mouse against twice the dose of acetonitrile lethal for a normal mouse. This dose is designated as 1 acetonitrile unit.

2. *The effect of thyroid gland on frog and salamander larvæ.* In some experiments in which preparations of various organs were given to tadpoles Gudernatch(4) in 1912 observed that the ingestion of thyroid tissue caused the growth of the tadpole to be arrested at once, and that metamorphosis took place very soon after the feeding and much earlier than normally, so that dwarf frogs would develop in this way.

This interesting phenomenon has been studied thoroughly by a number of investigators, among others, by C. O. Jensen(5); it has been used by Romais(6) and, later, by Rogoff(7) as the basis for a method of standardization of thyroid preparations. The method consisted in determining the smallest dose capable of eliciting the metamorphosis of a batch of tadpoles after one single feeding. The effect of each preparation was compared with the effect of a "standard preparation" given in the same dose. On comparison with control batches fed on liver it was possible to estimate roughly how many times stronger or weaker the unknown preparation was than the "standard preparation."

The experiments by Romais and by Rogoff showed that some of the commercial preparations were completely inactive, and that the active preparations varied several hundred per cent. in strength. The method then can really give valuable information, it is true, but it is hardly practicable, partly because one cannot make sure that the different batches of tadpoles get the same amount of food nor prevent those individuals that have had a small dose from eating more than their intended share, and partly because tadpoles are available only during a short part of the year.

A considerably better basis for a standardization offered when experiments by Laufenberger(8) and by C. O. Jensen(5) showed that the action of thyroid in amphibian larvæ not only promotes the metamorphosis but actually forces it; for the axolotl, in which the larval stage normally persists throughout life including breeding periods (neotenia), could be brought to metamorphosis at any time by administration of thyroid tissue. C. O. Jensen showed, too, that neotenia in the axolotl is due to a hereditary degeneration of the thyroid which takes place just at the period when metamorphosis normally should take place.

As the effect of thyroid feeding would probably be to furnish a substitute for the degenerated gland, C. O. Jensen(9) elaborated an

axolotl-method for quantitative determination of the active substances in thyroid preparations; he determined the smallest amount which injected into the peritoneum was able to produce the metamorphosis from gill-breathing water-animal to lung-breathing land-animal.

Later C. O. Jensen's method has been elaborated further by Schmit-Jensen(10); the effect of preparations is compared with the effect of thyroxine which, as shown by C. O. Jensen(11), has fundamentally the same, only a much stronger action on metamorphosis in the axolotl. In parallel series of experiments, with varying doses of thyroxine and dried gland respectively, the smallest dose of each of the two substances is found which is able to force the metamorphosis. The proportion between the values obtained is used as an indication of how many parts of glandular substance give the same effect as one part of thyroxine. The amount which in the axolotl experiment corresponds to 1 mg. of thyroxine is designated as 1000 "hormone" units.

3. *The effect of thyroid gland on the metabolism of warm-blooded animals.* The Danish physician Vermehren(12) was the first to demonstrate that thyroid gland influences metabolism in man, and Schøndorff showed that the metabolism of normal animals also is increased on its administration. This metabolic effect can be demonstrated in a number of functions: respiratory, nitrogenous, and carbohydrate metabolism, the regulation of temperature, elimination of water and sodium chloride, and other vital processes. Metabolism, respiratory and nitrogenous, has especially been used to demonstrate the effect. Abelin(13) has experimented extensively on thyroidectomized dogs and on rats, also Cramer and McCall(14), Hildebrand(15), and Arnoldi(16), without any attempt to elaborate from their observations a method of standardization. Recently Haffner and Komiyama(3) have investigated the effect of small doses of thyroid on the metabolism in white mice, with standardization in view, but they did not succeed in attaining a satisfactory method with their technique.

Pick and Pineles(17) have tried to determine the effect of different thyroid preparations on thyroidectomized goats; when the animals were definitely myxœdematous, they were given different thyroid preparations to try the effect of these on the disease.

Finally, it should be mentioned, that Streuli, and Duran(18) have attempted a standardization by making use of the observation that animals fed on thyroid are more sensitive to lack of oxygen than are normal animals, and Freud and Nobel(19) by simply determining the minimal lethal dose for guinea-pigs.

In working out a method for standardizing a drug it is first of all desirable that the property that is standardized should be that of which therapeutic use is made; the accuracy with which this property can be estimated should then be determined.

The therapeutic use of thyroid gland is always associated with its effect on metabolism, and, for this reason, determination of metabolic activity, more precisely respiratory exchange, is commonly practised in clinics. Hunt and Seidell, and also Haffner and Komiyama assume that the acetonitrile test gives a measure of an effect on metabolic activity; they think that the toxicity of acetonitrile depends on a cleavage of this substance in the organism whereby hydrocyanic acid is set free, and that thyroid gland causes a change (increase) in the metabolism so that no HCN is formed; they do not take into account the possibility of a direct combination of acetonitrile with constituents of the gland. There is nothing to prove that Hunt's view is correct; against it are the facts: (1) that it is *only in white mice* that the resistance to acetonitrile can be increased by ingestion of thyroiodin, while *all animals* show increase of metabolism on thyroid ingestion; (2) that iodothyrin (Baumann) is effective in the acetonitrile experiment but cannot protect animals that have been thyroidectomized after Pick and Pineles' method (Storm van Leeuwen); and (3) that the acetonitrile method gives values which correspond to the iodine content of the preparations, while there is no correspondence between this and their power to increase the metabolism.

As to the axolotl method, it undoubtedly indicates a direct thyroid action. For it is possible to prevent the metamorphosis of young tadpoles by removing their thyroid *anlage*, and it is possible by administration of thyroid to force metamorphosis in the axolotl which normally does not metamorphose. The method then seems to be well founded, but it is not in general use, probably because of the difficulty and expense of obtaining the very large number of animals necessary; for the minimal dose necessary to call forth metamorphosis is curiously not proportionate to the weight of the animal, so that it is essential to use animals of exactly the same weight; and, furthermore, it cannot be assumed that the specific thyroid action, which induces metamorphosis in the axolotl, is necessarily identical with that which increases metabolic rate in man.

It is generally recognized that a method of standardization should be based upon the rate of metabolism, and the method proposed by Pick and Pineles therefore would be ideal if it was possible to obtain constant values with it. But individual differences in the reaction of

thyroidectomized animals to thyroid administration are too great. Freud and Nobel's method was also found *not* to give applicable results, so that none of the methods hitherto proposed can be considered satisfactory.

II.

In connection with some investigations which did not directly bear on the standardization of thyroid preparations, it became necessary to use such a preparation, having a constant effect on the metabolism in white mice, and therefore to work out a method of standardization for this purpose. At that time the experiments by Haffner and Komiyama had not been published.

That the respiratory metabolism is increased in small animals (mouse, rat, dog) when fed on thyroid, as it is in human beings, was shown by Abelin and others. The respiratory quotient at the same time rises. Thus a determination of the carbon dioxide output in thyroid treatment will give the largest deviation from normal figures, and as besides it is readily estimated, it was decided to use the increase of carbon dioxide output as a measure of the effect of the different preparations.

The accuracy of a determination of respiratory metabolism depends largely on the experimental conditions. The following factors are of particular importance: (1) constancy in composition of the food, (2) activity of the animals' movements during the experiment, and (3) temperature. Uniform results can be obtained only when these three factors are controlled as far as possible.

A standard diet was used, consisting of a mixture of crushed barley 200 g., crushed corn 200 g., and milk-powder 80 g., and it was given in such amount (4 g. per 20 g. of mouse) that the animals always ate it up and throve upon it. It is more difficult to regulate variations in muscular activity, since experiments under anæsthesia are not suitable to the purpose. The consequence of this, however, can be eliminated, at least in part, by extending the experimental time over a longer period. As preliminary experiments showed that constant values are not obtained in less than 12 hours, a period of 24 hours was used in these experiments. The daily output of carbon dioxide is determined on several consecutive days (usually 5–6), so that the animals become accustomed to the respiratory chamber and keep very quiet. The food is placed in the chamber with the animals, the determination of "basal" metabolism being impracticable.

The influence of temperature on the metabolism of the mouse, which also is demonstrated by Aszodi[20], is evident from Table I.

TABLE I.

Weight of mouse	Production of CO_2 in 24 hours in g.				
g.	17°	20°	23°	26°	29°
14·5	3·00	2·91	2·75	2·64	2·51
8·0	2·19	2·10	2·05	1·90	1·70
18·0	3·35	3·18	2·95	2·84	2·68

The figures given are the mean value of five consecutive experiments.

As the table shows, the production of carbon dioxide depends largely on the experimental temperature; between 17° and 29° the carbon dioxide output diminishes considerably with rising temperature. It was decided to carry out all experiments at 23°, because it was found that the mice began to show signs of over-heating (moist coat) above this, while they became very restless at temperatures below 20°. To keep the temperature constant the respiratory chambers were placed in a water-bath kept at 23° with an accuracy of $\pm$ 0·1°.

The experiments were carried out as follows:

For one week preceding the determination of the metabolism the mice were put upon the standard diet mentioned above, receiving 4 g. per 20 g. body-weight + 3 c.c. of fresh milk daily; then each mouse was placed in its own respiratory chamber together with the day's ration, and the chambers (as many as 6) were placed in the electrically heated (23°) and regulated water-bath. An even current of air freed from carbon dioxide (8 l. per hour) was sucked through the respiratory chambers by means of a suction pump. The carbon dioxide expired by each mouse in 24 hours was absorbed in 200 c.c. of 1·5/*N* KOH and determined by

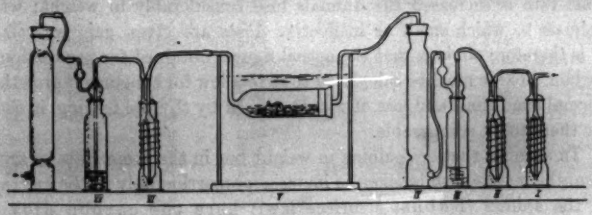


Fig. 1.

titration according to the method of Winkler. Fig. 1 shows the respiration apparatus.

The ingoing air was freed from carbon dioxide in the wash-bottles I and II containing strong solution of potassium hydroxide; it then passed through lime water coloured with phenol-phthalein in wash-bottle III to give warning of ineffective absorption in I and II. The air was then drawn from the reservoir (IV) into the respiration chambers (V), cylindrical glasses with inlet and outlet tubes, and a capacity of about 300 c.c. The outgoing air passed through the absorption bottles (VI and VII) containing KOH and out through a reservoir.

The accuracy attained in determining the output of carbon dioxide under these experimental conditions is evident from Table II, which shows that determinations made on the same animals deviate less than 7 p.c. from the mean value: in most instances the deviation does not exceed 5 p.c. The carbon dioxide output in different animals of the same weight, on the other hand, varies sometimes considerably more.

TABLE II. Normal mice.

Production of CO_2 in 24 hours on consecutive days in g.

No.	Weight in g.	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	Average
15	13·0	2·68	2·58	2·54	2·50	2·64	2·45	2·53	2·43	2·54
2	14·0	2·71	2·75	2·75	2·62	2·62	2·66	—	—	2·68
88	15·0	2·94	2·76	2·89	2·76	2·84	—	—	—	2·84
3	16·5	3·08	3·10	3·06	2·90	2·98	2·87	—	—	2·99
4	17·0	3·11	3·03	3·07	2·98	3·08	2·96	—	—	3·04
68	18·0	3·34	3·16	3·18	3·18	3·38	—	—	—	3·25
6	19·0	3·06	3·11	2·89	3·08	2·96	2·92	—	—	3·00
16	21·0	3·20	3·11	3·42	3·48	3·11	3·38	3·20	—	3·27

CALCULATION OF CARBON DIOXIDE PRODUCTION.

The carbon dioxide production of an animal is always changed when its weight changes. When large doses of thyroid are given and the metabolic rate is increased the animals lose considerably in weight; while animals to which small or ineffective doses are given gain in weight. It is therefore obvious that the actual figures obtained for production of carbon dioxide must be calculated so as to allow for concurrent growth in normal conditions and loss of weight caused by thyroid feeding, in order for them to be comparable.

In animals that are gaining in weight but in the same state of nutrition, the metabolism is taken to increase proportionately to the surface of the animal (Rubner's surface law); here the carbon dioxide production per square cm. of surface can be used as the unit. The surface area of the mouse can be estimated by Meeh's formula:

$$A = k.W^{\frac{2}{3}},$$

where A = area, W = weight, and k = a constant. Carman and Mitchell[21] have calculated k for rats as = 11·36; the same value applies undoubtedly to mice, as they have the same shape as rats.

Other laws seem to apply to animals that are losing weight. Rubner states that metabolism in starving animals decreases proportionately to weight. Abelin also and Haffner and Komiyama consider that the metabolism is changed proportionately to the weight, and they use the carbon dioxide production per gram of mouse as unit.

A series of experiments was done to settle this question.

Table III gives the experimentally obtained CO_2 values for a series

TABLE III. CO_2-production in 24 hrs. in normal mice.

			Observed			Calculated	
No.	Weight (g.)	Area of skin (cm.²)	Total* amount (g.)	Mg. per g.	Mg. per cm.²	Total amount from weight (g.)	Total amount from area (g.)
15	11·0	56·2	2·15	195·5	38·3	—	—
,,	13·0	62·9	2·49	—	—	2·54	*2·41*
,,	15·0	69·0	2·65	—	—	2·93	*2·64*
17	13·0	62·9	2·89	222·3	45·9	—	—
,,	15·0	69·0	3·16	—	—	3·33	*3·16*
,,	17·5	76·5	3·57	—	—	3·89	*3·51*
3	16·5	73·5	2·81	170·3	38·3	—	—
,,	19·0	80·9	2·98	—	—	3·24	*3·08*
,,	20·0	83·7	3·18	—	—	3·41	*3·20*
6	17·0	75·1	2·81	165·3	37·4	—	—
,,	19·0	80·9	3·00	—	—	3·14	*3·02*
,,	20·0	83·7	3·08	—	—	3·30	*3·13*
7	18·0	78·0	3·03	168·3	38·8	—	—
,,	21·0	86·5	3·37	—	—	3·54	*3·36*
,,	22·5	90·5	3·59	—	—	3·80	*3·51*
86	15·0	69·0	2·55	170·0	37·0	—	—
,,	14·0	66·0	2·42	—	—	*2·38*	2·44
,,	13·0	62·9	2·22	—	—	*2·21*	2·32
92	18·5	79·4	3·08	166·5	38·8	—	—
,,	16·2	72·6	2·70	—	—	*2·70*	2·82
93	19·0	80·9	3·16	166·3	39·1	—	—
,,	17·0	75·1	2·89	—	—	*2·83*	2·94
,,	15·4	70·2	2·53	—	—	*2·56*	2·74
94	19·0	80·9	2·92	153·7	36·1	—	—
,,	17·3	75·9	2·58	—	—	*2·66*	2·70
,,	14·5	67·4	2·24	—	—	*2·23*	2·44
96	19·3	81·7	2·78	144·0	34·0	—	—
,,	17·6	76·8	2·52	—	—	*2·54*	2·61
,,	14·6	67·7	2·07	—	—	*2·10*	2·30
97	24·0	94·5	3·76	156·6	39·8	—	—
,,	23·0	91·9	3·44	—	—	*3·60*	3·68
,,	22·0	89·2	3·31	—	—	*3·44*	3·56
,,	18·0	78·0	3·00	—	—	*2·82*	3·10

* Average of five consecutive experiments.

of normal mice, partly during increase of weight, partly during loss of weight in comparison with the values calculated on the basis of the animals' original surface area and weight respectively.

It is evident from the table that it is possible to calculate with almost astonishing accuracy how much carbon dioxide a mouse normally will produce at any time later on, no matter whether it gains or loses weight. If the mouse is gaining in weight, the carbon dioxide production increases proportionately to the surface of the animal; then the constant value, the carbon dioxide production (mg.) per cm.² of surface, is used as the basis for the calculation of the increase. When the mouse is losing weight, the carbon dioxide production is decreased proportionately to the weight; then the carbon dioxide production per g. of weight is used instead. When calculated in this manner, the experimentally observed values in Table III are in accordance with the calculated values. Table IV gives the surface in cm.² for mice weighing between 15 and 22 grams.

TABLE IV.

Weight (g.)	Area of skin (cm.²)	Weight (g.)	Area of skin (cm.²)
15·0	69·07	19·0	80·88
16·0	72·14	20·0	83·7
17·0	75·1	21·0	86·48
18·0	78·02	22·0	89·18

Thus it is possible to calculate the amount of any increase resulting from thyroid feeding, even when the carbon dioxide production might normally have been changed on account of change in weight. This is illustrated by the following examples:

Example 1.

	Weight (g.)	Surface (cm.²)	Total CO_2-production (g./24 hrs.)	Mg. CO_2 per cm.²
Normal value	18·7	80·0	3·25	40·6
After 0·1 mg. thyroid daily for 3 weeks ...	20·0	83·7	3·39	40·5
			Decrease: 0·1 = 0·25 %	

Example 2.

	Weight (g.)	Surface (cm.²)	Total CO_2-production (g./24 hrs.)	Mg. CO_2 per cm.²
Normal value	15·5	70·6	2·79	39·5
After 0·15 mg. thyroid daily for 3 weeks ...	18·5	79·4	3·48	43·8
			Increase: 4·3 = 10·9 %	

Example 3.

	Weight (g.)	Total CO_2-production (g./24 hrs.)	Mg. CO_2 per g.
Normal value	17·8	3·05	171·4
After 0·8 mg. thyroid daily for 3 weeks ...	14·5	3·69	255·8
		Increase: 84·4 = 49·2 %	

Effect of thyroid feeding on CO_2-production.

A single dose of thyroid by mouth is not sufficient to cause a demonstrable increase of the carbon dioxide production; a subcutaneous injection causes a decreased production within 1–2 hours after the injection, followed by a merely transitory increase; for an effect that can be measured it is necessary to administer the gland for several consecutive days. As for therapeutic purpose it is always given by mouth, this mode of administration is obviously also the most appropriate for the estimation of its therapeutic value. In all the experiments, therefore, the preparations were mixed with the food which was served in a vessel of such a construction that nothing was wasted.

It is in the next place necessary to ascertain at what time (that is, after how many daily doses) the increase of metabolism should be determined. In their experiments Haffner and Komiyama estimated it after feeding the gland to mice for a period of 5–10 days; the curve of the carbon dioxide production was then on the rise, and, as might be expected, seeing that the selection of this period was entirely arbitrary, they did not obtain figures that could be used for quantitative estimation of potency. Their experiments indicate merely that thyroid for this purpose must be fed to mice for more than 10 days. Observations extending over a longer period are not to be found in the literature.

Three possibilities present themselves: either (1) metabolism will increase continuously until the death of the animal (cumulative effect); or (2) metabolism will increase at first, but, as the organism gets accustomed to the preparation, the effect will after a time diminish, unless the dose is increased; or (3) for every dose there will be a corresponding definite increase of metabolism, and the maximal level will be maintained as long as the thyroid administration is continued. If the effect on metabolism proceeds as mentioned under (1), then any standardization on this basis is impossible. If the effect is as given under (2), the increase of metabolism should be determined at its maximum, and the method must fail unless this maximum sets in at the same time with large doses of thyroid as with small doses. If the effect is that mentioned under (3), the determination of carbon dioxide production, when this has become constant after feeding thyroid for some time, should furnish an excellent means of standardizing the drug. The following series of experiments was carried out in order to settle this question.

A number of mice were fed on the usual standard diet for 8 days prior to the experiment and then the carbon dioxide output was deter-

mined on each of five consecutive days; from the average the "normal value" was calculated. Next the animals were fed for 1–3 months with dried thyroid gland (preparation A) in daily dosage varying from 1·50 to 0·10 mg. per 20 g. of mouse. During the thyroid feeding the carbon dioxide production was determined at suitable intervals, and each time on from 3 to 5 consecutive days. Tables V and VI give the results of some of these experiments. In these Tables the words in the first column "After thyroid for 8 days" (or any other number of days) means that the determinations, from which the average rate appearing in col. 3 was calculated, were done on from 3 to 5 consecutive days, the last of which was the 8th day since administration of thyroid began. Early in the experiment three determinations were generally done, later, for instance after the first four weeks, generally five.

TABLE V.

	Weight (g.)	Total CO_2-production (g./24 hrs.)	CO_2-production mg. per g. weight	Increase of CO_2-production (%)
Mouse No. 18. 1·50 mg. thyr. gland daily per 20 g. weight:				
Normal value	14·0	2·29	163·6	—
After thyroid for 2 days	14·0	2·65	189·3	15·7
„ 10 „	14·0	3·44	245·7	50·4
„ 16 „	13·3	3·23	242·9	48·4
„ 30 „	14·0	3·47	247·9	51·5
Mouse No. 29. 1·00 mg. thyr. gland daily per 20 g. weight:				
Normal value	18·7	3·09	165·2	—
After thyroid for 8 days	16·5	3·26	197·6	19·6
„ 12 „	14·8	3·49	235·8	42·7
„ 20 „	14·8	3·61	244·0	47·7
„ 27 „	14·7	3·58	243·5	47·4
„ 50 „	15·3	3·67	239·9	45·2
Mouse No. 31. 0·80 mg. thyr. gland daily per 20 g. weight:				
Normal value	12·5	2·31	184·5	—
After thyroid for 8 days	12·5	2·56	204·8	11·0
„ 14 „	12·0	3·12	260·0	40·9
„ 28 „	12·5	3·27	261·6	41·8
Mouse No. 21. 0·65 mg. thyr. gland daily per 20 g. weight:				
Normal value	17·1	2·83	165·5	—
After thyroid for 5 days	16·0	2·83	176·9	6·9
„ 18 „	16·3	3·73	228·8	38·2
„ 32 „	16·3	3·67	225·2	36·1
„ 55 „	16·3	3·71	227·6	37·5

Table V comprises experiments in which the animals got rather large doses (1·50–0·65 mg.) and, on that account, lost weight; here the increase in carbon dioxide production is calculated per gram of body-weight. Table VI gives experiments in which the animals gained in weight, in

spite of the thyroid administration; here the increase of metabolism is calculated per cm.² of surface.

TABLE VI.

	Weight (g.)	Area of skin (cm.²)	Total CO_2-production (g./24 hrs.)	CO_2-production mg. per cm.²	Increase of CO_2-production (%)
Mouse No. 30. 0·40 mg. thyr. gland daily per 20 g. weight:					
Normal value	13·0	62·3	2·36	37·6	—
After thyroid for 8 days	13·5	64·3	2·63	40·9	8·8
„ 16 „	15·5	70·6	3·28	46·5	23·7
„ 30 „	14·2	66·6	3·18	47·7	26·9
Mouse No. 20. 0·25 mg. thyr. gland daily per 20 g. weight:					
Normal value	13·9	65·6	2·71	41·3	—
After thyroid for 5 days	15·6	70·9	2·99	42·2	2·2
„ 18 „	16·0	72·1	3·43	47·6	15·3
„ 30 „	18·0	78·0	3·71	47·6	15·3
„ 40 „	18·0	78·0	3·71	47·6	15·3
„ 50 „	17·3	76·9	3·64	47·3	14·5
„ 70 „	20·5	85·0	4·10	48·2	16·7
„ 90 „	19·0	80·8	3·85	47·6	15·3
Mouse No. 28. 0·20 mg. thyr. gland daily per 20 g. weight:					
Normal value	16·3	72·9	3·00	41·2	—
After thyroid for 14 days	19·0	80·8	3·70	45·8	11·2
„ 22 „	19·5	82·3	3·85	46·8	13·6
Mouse No. 22. 0·15 mg. thyr. gland daily per 20 g. weight:					
Normal value	16·0	72·1	2·75	38·1	—
After thyroid for 14 days	17·1	75·3	3·12	41·4	8·7
„ 22 „	17·8	77·4	3·20	41·4	8·7
Mouse No. 68. 0·10 mg. thyr. gland daily per 20 g. weight:					
Normal value	18·7	80·0	3·25	40·6	—
After thyroid for 20 days	20·0	83·7	3·39	40·5	−0·25
„ 30 „	21·0	86·5	3·52	40·7	0·25
„ 50 „	24·0	94·5	3·82	40·4	−0·5

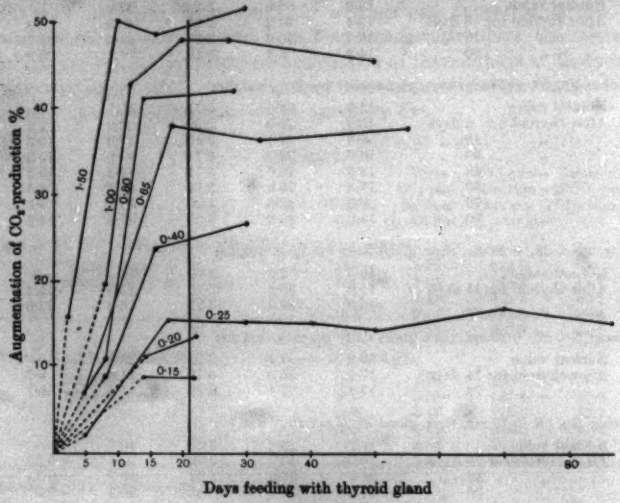
The result of the experiments is presented graphically in Curve I. It was, briefly, as follows:

(1) The carbon dioxide production (metabolism) is increased only up to a certain point with continuous daily administration of the same dose of gland; a cumulative effect then is hardly likely.

(2) When the increase of metabolism has reached its maximum for the given dose of thyroid, the carbon dioxide production remains constant as long as the thyroid administration is continued (see mouse No. 20, Table VI); no tolerance is established. This is in accord with clinical findings by Eggert Möller[22] in the treatment of myxœdema in human beings with thyroid gland.

(3) With very large doses of gland (1·00–1·50 mg.) the maximal increase of carbon dioxide production is obtained in 10–12 days, with smaller doses in 14–20 days. If 20 days' administration does not result in any increase of the carbon dioxide output, administration for a longer period will likewise prove ineffective.

(4) For a given daily dose of thyroid there is a corresponding definite increase of metabolism.



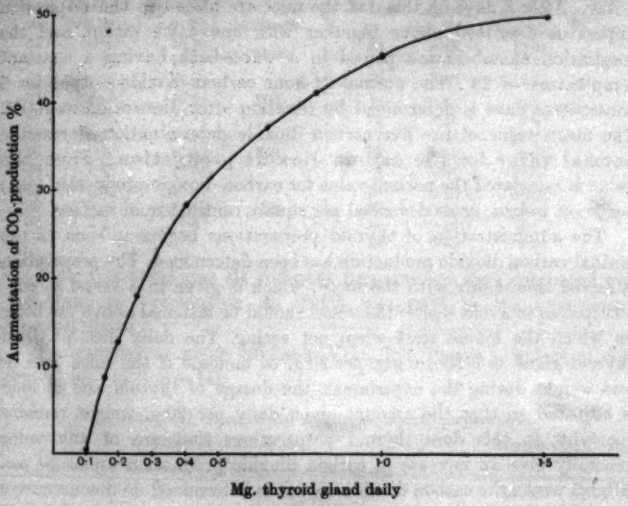
Curve I.

The experiments were repeated with three different thyroid preparations. In every case the result was that the increase of carbon dioxide production had reached its maximum for the given dose after 3 weeks' daily administration of the same dose of gland, and that this maximum kept constant for the remainder of the experimental period. In order to determine the effect on metabolism of a given dose of thyroid gland it is therefore sufficient to estimate the carbon dioxide production (1) before the thyroid feeding and (2) after 3 weeks' administration.

The effect of increasing doses of gland is proportional to the size

of the dose only within a certain limit. Consequently, if the effect of a given dose is to be used for standardization, it is essential to know exactly the relation between dose and effect.

Curve II ("curve of concentration effect") shows how the CO_2 increase in a given experimental series depends upon the amount (concentration) of thyroid gland; the amount of thyroid-containing substance is recorded as abscissa, and the CO_2 increase as ordinate. A given



Curve II.

thyroid concentration, however, does not produce an absolutely definite, corresponding, value of CO_2 increase. For the CO_2 increase may be different in different individual animals, and, with an increase as high as 30–35 p.c. or more, this variability is so great as to make the determination unreliable, since all animals do not reach the same level. But with an increase that is within the range of 0–30 p.c. a given percentage increase of the dose will produce a fairly constant increase of CO_2-production with comparatively slight individual variations, and any standardization should consequently be carried out within these limits. Comparison of various preparations would therefore be

satisfactory if a dose of thyroid gland were used which produces a CO_2 increase amounting to 15 p.c.

The technique for standardization of thyroid preparations to which these experiments have led is as follows: white mice, preferably males, weighing 16–22 g., are fed on the standard diet (crushed corn 200 g., crushed barley 200 g., dry milk 80 g.) in such an amount that the animals will keep their weight, or gain a little; 4 g. per 20 g. of mouse was found sufficient with the addition of 3 c.c. of fresh milk a day. After 8 days on this diet the mice are placed in the respiration apparatus described above together with one day's ration, and the respiration chambers are placed in a water-bath having a constant temperature of 23°. The normal 24-hour carbon dioxide output for 5 consecutive days is determined by titration after absorption in KOH. The mean value of the five carbon dioxide determinations forms the normal value for the carbon dioxide production. From this value is calculated the normal value for carbon dioxide production (mg.) per gram weight, or as described per square centimetre of surface.

The administration of thyroid preparations begins as soon as the normal carbon dioxide production has been determined. The preparation is mixed thoroughly with the food[1], which is given in a vessel so constructed as to avoid waste; the vessel should be fastened above the litter on which the mouse rests when not eating. The daily dose of dried thyroid gland is 0·10–1·0 mg. per 20 g. of mouse. If the mice gain or lose weight during the experiment, the dosage of thyroid and of food is adjusted so that the amount given daily per 20 g. weight remains constant; in this dose thyroid preparations that are of any value generally give an increase in carbon dioxide production up to 50 p.c. After 3 weeks the carbon dioxide output is determined on 5 consecutive days (24-hour eliminations), the mean value of these five determinations is computed, and the carbon dioxide production is calculated, if the animals have lost in weight, per gram of body-weight, if they have gained, in cm.^{2} of surface, as explained on p. 229. The standard for comparison of different preparations is that dose which causes the carbon dioxide production to increase 15 p.c. This dose is readily determined by constructing a curve from 4–6 values obtained experimentally.

In order to find out with what degree of accuracy the increase in carbon dioxide production can be determined which is caused by a given dose of thyroid, a series of mice were fed with 0·1–0·8 mg. per 20 g. of mouse, several animals getting the same daily dose. The result of this

[1] May also be given as a small pill.

experiment is given in Table VII. From this it is evident that it is possible to distinguish between the effect of a dose and of one 33 p.c. larger, and that the increase with the same dose in different animals deviates ± 15 p.c. from the mean value (mice Nos. 19 and 20). It is therefore safest to make duplicated determinations.

TABLE VII. Accuracy of standardization method. Glandula Thyreoidea sicca "A."

Mouse no.	Thyroid preparation A (mg./20 g.)	Increase in CO_2-production (%)	Average	Mouse no.	Thyroid preparation A (mg./20 g.)	Increase in CO_2-production (%)	Average
27	0·80	40·2	41·0	28	0·20	13·6	12·7
31	0·80	41·8		78	0·20	12·3	
21	0·65	38·2	37·1	79	0·20	12·1	
23	0·65	36·2		22	0·15	8·7	8·8
26	0·65	37·0		70	0·15	5·6	
24	0·40	29·3	28·7	71	0·15	10·9	
25	0·40	29·9		76	0·15	8·5	
30	0·40	26·9		77	0·15	10·3	
19	0·25	20·8	18·0	68	0·10	−0·25	0·3
20	0·25	15·3		69	0·10	1·3	
32	0·25	17·8		74	0·10	4·1	
				75	0·10	−4·2	

With this technique six different preparations were compared with the standard preparation A, which was used in the experiments reported above.

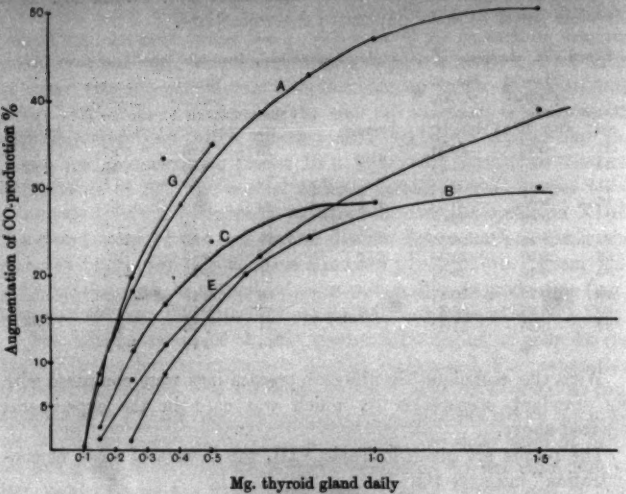
The results are given in Table VIII, the "curve of effect of concentration" in Curve III.

TABLE VIII. Comparison of different thyroid preparations.

Mouse no.	Dose	Increase in CO_2-production (%) In test	With A	Mouse no.	Dose	Increase in CO_2-production (%) In test	With A
Preparation B:				Preparation D:			
38	1·50	30·0	51·5	58	1·50	0·8	51·5
43	0·80	24·6	41·0	Preparation E:			
36	0·60	20·5	36·0	63	1·50	39·2	51·5
34	0·35	9·0	25·0	61	0·65	21·8	37·1
42	0·25	1·2	18·0	59	0·35	12·0	25·0
Preparation C:				60	0·25	8·0	18·0
56	1·00	28·4	47·7	62	0·15	1·0	8·8
47	0·50	24·1	32·2	Preparation G:			
54	0·35	16·7	25·0	85	0·50	34·7	32·2
53	0·25	11·4	18·0	84	0·35	33·5	25·0
55	0·15	2·3	8·8	83	0·25	19·6	18·0
Preparation D:				82	0·15	7·6	8·8
57	2·00	−3·1	—	80	0·10	0·9	0·3

Preparations A and G were furnished by Laboratory Director Th. Pedersen, Det Danske Medicinal- og Kemikaliekompagni, whom I thank.

It is evident from Table VIII and from Curve III that five of the preparations tested were capable of increasing the carbon dioxide production 15 p.c. on administration of a daily dose, which varied from



Curve III.

0·2 to 0·46 mg. per 20 g. of mouse, while one preparation had no effect even in the largest dose (2·0 mg. per 20 g. of mouse). Investigation of even this small number of preparations shows considerable differences in their value.

It is convenient to express the value of preparations in units per gram weight. It is proposed that the unit should be that amount (by weight) which, when given by mouth to white mice daily for 3 weeks and calculated per 1000 g. of mouse, is able to elicit a 15 p.c. increase in the carbon dioxide production.

Table IX gives the strength of the six preparations expressed in thyroid units, together with various analytical data (iodine content, percentage of water and ash). As shown, two of the preparations contained ca. 100 units, 1 ca. 65 units, and 2 ca. 45 units per gram, while one preparation contained less than 10 units per gram and, consequently, must be characterized as worthless.

TABLE IX.

Thyroid prep. marked	H_2O %	Ash %	Iodine %	Dose of thyroid causing 15 % increase of CO_2-production		Thyroid units per g. preparation
				mg. per 20 g.	mg. per 1000 g.	
A	10·9	11·4	0·20	0·22	11	91
B	12·5	3·0	0·19	0·46	23	43
C	9·1	4·6	0·35	0·32	16	63
D	8·6	3·8	0·23	>2·0	>100	<10
E	4·0	5·8	0·30	0·44	22	45
G	5·8	3·5	0·35	0·20	10	100

No parallelism is found between iodine content and effect on metabolism; on the contrary, the ineffective preparation contained more iodine than did one of the most effective (Prep. A). Thus the necessity of a biological method of standardization is confirmed.

SUMMARY.

A standard method for determination of the value of thyroid preparations has been worked out based upon the determination of the carbon dioxide production in white mice, at first under normal conditions (feeding with "standard diet"), then after administration of the preparation.

The optimal conditions for this determination (temperature, experimental period "standard diet") have been established. The optimal temperature was found to be 23° C. (Table I), as the animals at higher temperature showed sign of overheat while they became restless at lower temperatures; the experimental period was fixed at 24 hours, as less than 12 hours gave inconstant values; the "standard diet" used was a mixture of crushed barley, corn and milk-powder. The accuracy attained in determining the carbon dioxide production on these experimental conditions is evident from Table II.

The carbon dioxide production of an animal changes when the weight is changed, and as the weight of animals fed with thyroid often alters during the experimental time (loss of weight with large doses, gain in weight with small) it is obvious that the absolute carbon dioxide values cannot be used for calculating the effect; the normal values as well as the values obtained after thyroid feeding must be recalculated to a common scale. It has been shown (Table III) that the carbon dioxide production increases proportionally to the area of the surface of the animal when the mouse is gaining in weight, but decreases proportionally to the weight of the animal when the animal is losing in weight. Thus the increase of CO_2-production due to thyroid feeding

if the animal is gaining in weight must be calculated per cm.^{2} area, if it is losing weight per g. body-weight. Table IV gives the area of the surface of mice of 15–22 g.

Feeding daily the same dose of thyroid gland to white mice causes an increase of the carbon dioxide production, which reaches its maximum at the earliest in 10 days (with large doses) at the latest in 3 weeks; each dose corresponds to a definite increase of metabolism; when the maximal increase for the dose is attained, this level persists as long as the thyroid is given (Tables V and VI, Curve I). It is therefore sufficient to estimate the carbon dioxide production (1) before the thyroid feeding and (2) after 3 weeks' administration.

The accuracy of the method is demonstrated in Table VII; it is possible to distinguish with certainty between the effect of one dose and that of another that is 33 p.c. larger.

The most accurate results in determining the increase of CO_2-production are obtained with doses of thyroid that give 0–35 p.c. increase (Curve II). The comparison of different preparations is by reference to a standard thyroid unit, the unit being that amount (by weight) of thyroid preparation which, when given by mouth to white mice daily for 3 weeks and calculated per 1000 g. of mouse, is sufficient to cause a 15 p.c. increase of the carbon dioxide production.

Estimation of six different preparations of *Glandula thyreoidea sicca* demonstrates the necessity of a standardization as the strength of the preparations varied from less than 10 to 100 thyroid units per gram (Tables VIII and IX, Curve III). It is evident from Table IX that there is no parallelism between the iodine content of the preparations and their effect on metabolism; a biological method of standardization is therefore necessary.

Hunt and Seidell's acetonitrile method gives values which are constantly proportional with the iodine content of the preparations. This method therefore gives hardly any information of the therapeutic value of the preparations.

It may be that C. O. Jensen's axolotl method gives values which indicate the content of therapeutically active substances in thyroid preparations; a comparison of the results from these two methods of standardization is desirable on this account.

The accuracy of the method presented is adequate for practical purposes. The necessary experimental animals are readily obtained and inexpensive. The technique of the method is extremely simple to one who is at all familiar with biological experiments.

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THE EFFICIENCY OF BICYCLE-PEDALLING, AS AFFECTED BY SPEED AND LOAD.

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It has been shown both for the case of isolated frog's muscle and for certain human muscular movements(1, 3, 4) that the work done in a maximal contraction is diminished by increase of rate of shortening. The relation between the work done and the speed of shortening is found to be linear, so that the relation between the work W and the time t occupied by the contraction is given by the equation

$$W = W_0\left(1 - \frac{k}{t}\right) \qquad \text{.....(1),}$$

where W_0 and k are constants. W_0 represents the theoretical maximum work and would be attained only if the contraction could take place infinitely slowly. k represents the theoretical minimum time and would be attained only if no external work were done. From experiments on isolated frog's muscle a relation has been found between the theoretical maximum work W_0, the energy H liberated in an isometric contraction excited by a maximal stimulus, and the time t during which the stimulus lasts. It is given by the equation

$$H = W_0 a\,(1 + bt) \qquad \text{.....(2),}$$

where a and b are constants: a represents the energy required to set up a contraction capable of doing one unit of work under maximal conditions: the product ab represents the energy required per second of stimulus to maintain that contraction. From equations (1) and (2) it can be deduced(5) that the mechanical efficiency E of a muscular movement will be given by the equation

$$E = \frac{1 - (k/t)}{a\,(1 + bt)} \qquad \text{.....(3).}$$

[1] Working for the Industrial Fatigue Research Board of the Medical Research Council.

If equation (3) holds for any muscular movement then the efficiency would be independent of the force overcome, but would vary with change of the time occupied by the movement. The efficiency would tend towards zero for times given by $t = k$ and $t = \infty$ and would pass through a maximum value (1, 6) for a time of contraction given by

$$t = k\left(1 + \sqrt{1 + \frac{1}{bk}}\right).$$

The way in which efficiency varies with time of contraction has already been determined in the case of isolated frog's muscle (6), and for certain types of human muscular movements (2, 7, 8). In a previous paper (4) it was shown that the relation expressed by equation (1) holds for the movement of bicycle-pedalling, and it was predicted that the efficiency of bicycle-pedalling would vary with the speed and that there would be an optimum speed at which the efficiency is highest. In the present investigation the efficiency of bicycle-pedalling was determined at various speeds.

Experimental procedure. Determinations were made of the total amount of oxygen consumed by a subject during exercise and recovery[1], as the result of the performance of a known amount of work on a bicycle ergometer working against a given load at a given rate of pedalling. A Martin's friction ergometer was used. The arrangement adopted, the dimensions of the bicycle, the method of recording the number of turns and the rate of pedalling are described in a previous paper (4). For the measurement of some of the smaller loads a weight was hung at one end of the friction band directly over the bicycle wheel, and a more sensitive spring-balance was used at the other end.

A system of Douglas bags, and a Haldane gas-analysis apparatus were employed. The bags (9, p. 87) were supported on a table at the side of the bicycle. A tube from the expiratory side of the mouthpiece worn by the subject led to a three-way tap fixed on the handle-bars, and from this a tube led to a pipe connected with the system of bags.

Collections of expired air were made: (1) over a period of ten minutes immediately after the subject had remained at rest on the bicycle for half an hour; (2) over a period of half an hour, the first one to ten minutes of which (according to the speed of pedalling) were occupied by the exercise and (3) over a period of ten minutes immediately after the half-

[1] It was necessary to employ the method of measuring the oxygen "requirement" in this way, since a steady state cannot be attained at the higher rates of working, and therefore a measurement merely of the oxygen consumption during the exercise would give fallacious results.

hour period. From the first and third collections was determined the oxygen used per minute when sitting at rest on the bicycle immediately before the exercise and after recovery. From the second collection the total oxygen used during the period of exercise and recovery was found. The oxygen used for the exercise was taken as the difference between the total amount used during the thirty-minute period and the amount that would have been consumed during that period at the mean of the rates of oxygen consumption found for sitting on the bicycle before and after that period. It was assumed that the foodstuff utilized for the excess metabolism of the exercise was glycogen, in which case the energy value of 1 litre of oxygen would be 5·14 calories (10, p. 6), which is equivalent to 2190 kilogram-metres of energy expended. Hence the efficiency E is given by the formula

$$E = \frac{Rnl}{V \times 2190},$$

where R is the load at the rim of the wheel expressed in kilograms, l is the circumference of the wheel expressed in metres, n is the number of wheel revolutions occurring during the exercise, and V is the number of litres of dry oxygen at N.T.P. used for the performance of the exercise.

Results.

The relation between efficiency and speed of movement. Table I shows a series of measurements of efficiency with different rates of pedalling. The load in all these measurements lay between 2·8 kg. and 3·4 kg. at the rim of the wheel, except in the case of the two measurements given at the end of the table in which the load was 5·2 kg. The force opposing the movement of the pedal is roughly 3·7 times the load at the rim of the wheel. The results are shown graphically in Fig. 1, in which the abscissa represents the time in seconds of one foot movement (half a pedal revolution) and the ordinate represents the efficiency expressed as a percentage. The continuous curve shown in the figure is obtained from the experimental points. It is seen from the graph that the efficiency is low for both high and low rates of pedalling, and that it passes through a maximum value of 21·5 p.c. at a time for one foot movement of about 0·9 second.

Two theoretical points may be considered in relation to this curve. (A) From experiments described in a previous paper (4) it was found for the subject of these experiments that the theoretical minimum time of one foot movement when there is no external load is 0·16 sec. A theoretical point can therefore be added to the curve corresponding to a time of movement of 0·16 sec. and zero efficiency. (B) Since the energy

TABLE I.

Resting oxygen consumption: c.c. per minute	Oxygen used during exercise and recovery: litres	Oxygen used for exercise: litres	Time of one foot movement: seconds	Load at rim of wheel: kg.	No. of turns of wheel	Work done: kg.-metres	Efficiency: p.c.
254 266	12·17	4·37	0.27	3·0	227	1090	11·4
279 278	13·33	4·98	0.46	3·1	400	1984	18·2
276 275	11·93	3·67	0.50	3·2	294	1505	18·7
277 279	12·75	4·41	0.63	3·1	400	1984	20·5
239 242	11·71	4·50	0.79	2·85	455	2074	21·0
262 276	12·68	4·61	0.78	3·2	396	2030	20·1
245 252	12·24 (31 min.)	4·55	0.90	3·4	400	2176	21·8
261 268	13·32	5·42	0.90	3·0	532	2554	21·5
242 242	11·20	3·94	1.11	2·75	400	1760	20·4
247 251	11·83 (31 min. 7 sec.)	4·38	1.20	3·05	400	1920	20·0
251 243	11·04	3·63	1.81	3·05	294	1411	17·7
273 267	12·89 (32 min.)	4·25	2.71	3·3	266	1405	15·1
267 ?	13·74 (33·5 min.)	4·80	3.62	3·2	271	1387	13·4
276 259	10·34	2·33	0.37	5·2	95	790	15·5
259 255	10·92	3·21	0.50	5·2	146	1214	17·3

Note. The time of exercise and recovery was 30 minutes except where otherwise stated.

required to maintain a muscular contraction increases with the time during which it is maintained, an infinitely slow contraction would require an infinite expenditure of energy. We may therefore suppose that the efficiency tends to zero as the time of movement becomes very great. That the efficiency should be zero at times $t = 0{\cdot}16$ and $t = \infty$ agrees reasonably well with the experimental points. The broken curve in Fig. 1 is a theoretical curve derived from equation (3) by giving to the constants the values $k = 0{\cdot}16$ sec., $a = 2{\cdot}8$ and $b = 0{\cdot}435$. It is seen from the figure that the experimental curve has the same general form as the theoretical curve.

The effect of load on efficiency. Table II shows a series of measurements of efficiency with varying load, but with the speed of pedalling

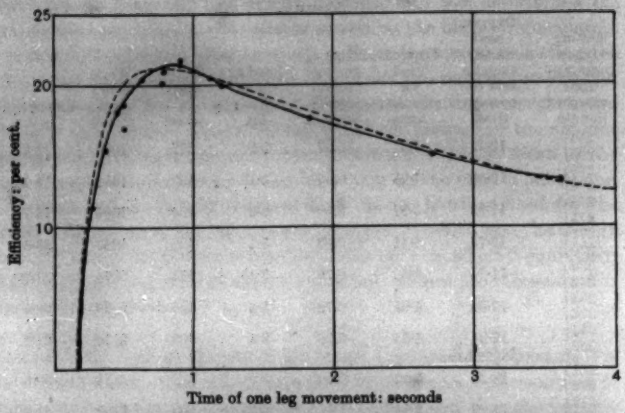

Fig. 1. The relation between E the efficiency and t the time of one leg movement. The plotted points represent the results of the experiments. The continuous curve is drawn through these points. The broken curve is a theoretical curve derived from the equation $E=\frac{1-(k/t)}{a(1+bt)}$ when the values of the constants are $k=0{\cdot}16$ sec., $a=2{\cdot}8$ and $b=0{\cdot}435$ per sec.

TABLE II.

Resting oxygen consumption: c.c. per minute	Oxygen used during exercise and recovery: litres	Oxygen used for exercise: litres	Time of one foot movement: seconds	Load at rim of wheel: kg.	No. of turns of wheel	Work done: kg.-metres	Efficiency: p.c.
229 / 235	10·513	3·55	0.90	1·15	930	1711	22·0
235 / 233	10·926	3·91	0.90	1·90	600	1824	21·2
261 / 268	13·32	5·42	0.90	3·0	532	2554	21·5
245 / 252	12·24 (31 min.)	4·55	0.90	3·4	400	2176	21·8
249 / 256	12·37	4·80	0.90	5·0	267	2136	20·2*
252 / 240	12·37	4·99	0.90	6·05	240	2323	21·4
256 / 264	11·91	4·10	0.90	7·2	160	1843	20·6*

* The two low values were both obtained on the same occasion soon after the subject had had influenza. The low efficiency might be due to unskilled use of muscles.

constant at the value found to give the maximum efficiency in the previous series of experiments. The loads given are those at the rim of the wheel. Thus the range of force investigated was from 5·2 kg. to 26·6 kg. opposing the movement of the foot. The investigations were limited to this range because (*a*) with smaller loads the subject could not keep time with the metronome without "holding the legs back," and (*b*) with greater loads the subject could not move the pedals smoothly over the "dead points" and there was a tendency to "stand on the pedals" and so alter the type of movement. It is seen from the table that within the range used change of load has no appreciable effect on the efficiency if the speed of pedalling remains constant.

This result confirms the application of equation (3) to submaximal movements: strictly speaking it was deduced only for maximal ones, but on the assumption that the force exerted is graded merely by varying the number of muscle fibres exerted it should apply also to the more general case. To make the investigation complete would theoretically require the determination of an efficiency-time curve for each load. The present determinations, however, are sufficient to demonstrate the absence or at least the comparative smallness of the effect of load when the speed is constant at the chosen value.

The oxygen consumption for unloaded movement. Two experiments were made to determine the energy cost of unloaded pedalling at two different speeds. The results are shown in Table III. As would be expected the cost is greater for the greater speed, but in both cases the value is higher than would be expected. It was found that when there was no load to work against it was very difficult to keep time with the metronome without using muscles to hold the legs back. It is probable, therefore, that the oxygen consumption found in an unloaded experiment is higher than that part of the oxygen consumption that is required for the work done in moving the legs themselves during a loaded experiment.

TABLE III.

Resting oxygen consumption: c.c. per minute	Oxygen used during movement and recovery: (15 min.) litres	Oxygen used for movement: litres	Time of one foot movement: seconds	Load	No. of turns	Work	Oxygen used per 100 turns: litres
258	4·35	0·54	0.82	—	266	—	0·203
250	4·50	0·70	0.48	—	262	—	0·268
258							

Discussion.

Previous work on the efficiency of human muscular movement. That the speed of limb movement has a marked effect on the efficiency of doing work against an external force, and that for any given type of movement there is an optimum speed at which the efficiency is a maximum, may be taken as satisfactorily established. Lupton for stair climbing(2) and Furusawa for arm movements working a lever(7) have found efficiency-time curves of the same general form as the one described in this paper for bicycle-pedalling. Atzler, Herbst, Lehmann and Müller(11) for the case of turning a crank with the arms have found a similar variation of efficiency with speed. Cathcart, Richardson and Campbell(8) from experiments on a "variable ergometer" using arm muscles, came to the conclusion that there was an optimum rate of movement for doing that type of work. For bicycle-pedalling Benedict and Cathcart(12, p. 140) found the highest efficiencies with the lowest rates of pedalling, but their experiments did not include any with very slow rates. In all cases in which a definite efficiency-time curve has been worked out it has the same general form as the one described here for bicycle-pedalling. The curve has a broad optimum zone so that it is not always possible to give exactly the optimum time, but it can be found to lie between certain limits.

The conclusions concerning the effect of load on efficiency drawn from various sources are not so definite as those concerning the effect of speed. The experiments described in this paper show no appreciable effect of load on efficiency when the speed of pedalling is constant at the speed chosen for those experiments. Furusawa(7) concluded from his experiments that change of load does not affect the energy expenditure for amount of work done at constant speed of movement and that there is no optimum load. Results given by McDonald and Duffield(13) show no effect of load on efficiency. On the other hand Cathcart(14) has found an increase of efficiency with increase of load from small to moderate ones and then a falling off of efficiency with further increase to excessive loads. This would seem to indicate the existence of an optimum load, but the differences found were small, especially as compared with those found for varying speeds. Experiments described by Atzler, Herbst, Lehmann and Müller(11) show the same effect as that found by Cathcart. Campbell, Douglas and Hobson(15) describe results that show a fall of efficiency with increase of load at constant speed. It would appear that the effect of load on efficiency at

constant speed is, if not negligible, at any rate small. Where an effect is found, and the experiments include a large enough range of loads starting from very small ones, there is first a rise of efficiency and then a fall with increasing load. This may be explained by the operation of two factors which have not yet been considered.

In the first place, no account has been taken of the energy expended in guiding and moving the limbs. Since, for a given speed, this becomes a smaller fraction of the whole energy when the load is made greater, this factor will cause an increase of efficiency with increasing load. A more accurate determination of the efficiency would be obtained if from the oxygen consumption found for the work there was subtracted that required for moving the machine without a load at the same speed. This cannot be done in practice for the reasons given above. Benedict and Cathcart in their work on bicycle-pedalling tried the experiment of taking as base-line the cost of unloaded movement and came to the same conclusion(12, p. 119). One way of overcoming the difficulty is to take as base-line the cost of movement when working at the required speed against a small load that is just sufficient to enable the movement to be performed without the tendency to "hold the legs back." Any two loads may be taken, and the efficiency calculated by dividing the excess work with the larger load by the excess energy. Since the experiments described here show no appreciable change of efficiency with change of load, therefore either the cost of movement alone is small and the efficiency is constant, or else the cost of movement is appreciable and there is a fall of efficiency with increase of load which balances its effect. The experiments described by Campbell, Douglas and Hobson(15) give an efficiency of the excess work with a larger load that is definitely lower than the net efficiency working with a smaller load. On the other hand, Benedict and Cathcart(12) found the efficiency of excess work taking a moderate load as base-line to be 30–33 p.c., some measurements giving a value as high as 40 p.c. It must be noted, however, that the larger load used by Campbell, Douglas and Hobson was greater than that used by Benedict and Cathcart, and that the speed of pedalling used by Campbell, Douglas and Hobson was smaller.

Taking into consideration this factor of the work done in moving the limbs alone the results may be interpreted as showing the existence of a second factor, a fall of efficiency with excessive loads. The two factors together would cause a rise and then a fall of efficiency with increasing load. The fall of efficiency with excessive loads may be due to the use of different muscles and of different types of effort to overcome them.

This is obviously the case in such movements as lifting a weight, or walking along pulling or pushing against a force, or walking uphill: while in bicycle-pedalling excessive loads may require the use of the arms to keep the body in the saddle, and may lead to difficulties in passing over the "dead points."

From these considerations it would seem that the experimental results are not fundamentally inconsistent with the view that the efficiency of uncomplicated muscular work is unaffected by change of the force overcome.

The maximum efficiency observed in the present experiments is lower than that found by previous workers for bicycle-pedalling (12, 15). This may possibly be due to the fact that the bicycle used was of dimensions suitable for a bicyclist of larger build than the subject of these experiments. The length of pedal crank most commonly used for bicycles is 6½ inches. This length is presumably adopted empirically as being suitable for a bicyclist of normal male build. The length of crank used in these experiments was 7 inches and the subject's height was only 5 ft. 3 in. The height of the saddle at its lowest point of adjustment was such that a considerable amount of extension of the ankle joint was required for the subject to reach the pedal at its lowest position. The experiments described, therefore, are not of value in supplying data as to the maximum efficiency attainable in bicycle-pedalling; their main object was to demonstrate the effect of speed and of load on efficiency.

The mechanical efficiency as measured in experiments on human limb movements is affected by the skill of the subject as well as by the mechanical conditions involved and by the efficiency with which a muscle does work. A skilled subject uses only those muscles required to perform the movement; an unskilled subject uses unnecessary muscles as well.

General theory. If the relation between factors involved in determining the efficiency of muscular work be adequately expressed by equation (3), then it follows that the efficiency at constant speed will be independent of the load and that an experimental efficiency-time curve will have the same general form as a theoretical curve derived from this equation. How far the experimental results are in accordance with this has been discussed in the previous section.

By an independent method (4) a value for the constant k was found for the subject of these experiments. The value found was 0·16 sec. Now it is seen from equation (3) that we may write $1 + bt = \frac{1}{a}\left(\frac{1-(k/t)}{E}\right)$,

so that if the equation holds the relation between t and $\frac{1-(k/t)}{E}$ should be linear. By taking the experimental value for k a series of values for t and $\frac{1-(k/t)}{E}$ can be found from the results of the experiments. These are shown graphically in Fig. 2. It is seen that the points are roughly

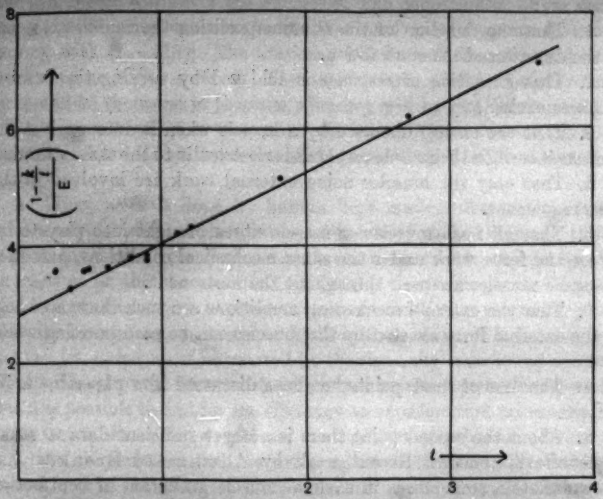

Fig. 2. The abscissa represents t the time of one leg movement. The ordinate represents $\frac{1-(k/t)}{E}$, where k is the "theoretical minimum time" and E is the efficiency, both experimentally observed. Note that the relation is linear, which demonstrates the validity of equation (3).

distributed about a straight line. Since when $t = 0$, $a = \frac{1-(k/t)}{E}$ and when $\frac{1-(k/t)}{E} = 0$, $b = -(1/t)$, values for a and b can be found from the graph. These are found to be $a = 2{\cdot}8$, $b = 0{\cdot}435$. These are the values of the constants used in drawing the theoretical curve shown by a broken line in Fig. 1. By direct measurement on arm muscles Lupton(2) found the values $a = 2{\cdot}6$ and $a \times b = 0{\cdot}486$. His determination of $a \times b$ was made from experiments in which a maximal contraction was maintained statically, so that whatever factors are introduced by actual movement and doing work were not involved.

Although it is of interest to note the order of magnitude of the constants a and b so determined, the results obtained cannot be taken as indicating precisely the values of these constants for human muscles, because in applying equation (3) to interpret the experimental results certain assumptions have been made which are not necessarily correct. These are:

1. That the duration of the stimulus exciting the muscles is equal to the duration of the contraction.

2. That gradation of response is achieved by varying the number of fibres excited.

3. That the energy liberated by a muscle when it does work is the same as it is when the muscle responds isometrically to the same stimulus.

4. That only the muscles doing external work are involved in the limb movement.

5. That all fresh muscles or muscle fibres brought into play to increase the force work under the same mechanical conditions, and that the same muscles are used throughout the movement.

6. That the external mechanical conditions are such that the whole of the external force exerted by the muscles can be used in doing work.

1. The first of these points has been discussed in a paper by A. V. Hill(6).

2. About the second point there is not yet sufficient data to make a definite statement. Recent work by Adrian and Bronk(16) has shown that in some cases in skeletal muscle gradation of contraction is brought about not only by variation in the number of fibres in action but also by variation of the frequency of the discharge along the nerves supplying them. If this is so, then in a submaximal contraction doing work against a given force at a given rate of movement the number of fibres involved will be greater than if the fibres were excited to a maximal contraction and the efficiency would be less. To discover to what extent this factor will effect the efficiency it is necessary to know the conditions of energy liberation in an incomplete tetanus. This point is at present being investigated here by Dr Bronk. It would appear from the results he has so far obtained, taken in conjunction with the actual range of frequency of impulses found to occur in the mammal, that the effect of this factor on efficiency would not be large.

3. As regards the liberation of energy by a muscle doing work it was shown by Fenn(17) that a muscle excited by a maximal stimulus liberates more energy if it does external work than if it is kept under isometric

conditions. The matter has recently been further investigated by Hartree and Hill(18). Since the excess energy due to work is a complicated function of the work and of the duration of the contraction, a simple method of taking account of this factor in discussing the efficiency cannot be given. It is possible, however, to see qualitatively how this factor will affect the efficiency. The denominator of the ratio giving the efficiency must be greater than $\{a(1+bt)\}$ by some quantity varying with $\{1-(k/t)\}$. The efficiency will be less than it would be if there were no "Fenn effect." The general form of the efficiency-time curve will be the same in that the efficiency will be zero for times $t=k$ and $t=\infty$ and will pass through a maximum. The values of the constants a and b deduced from the simple equation (3) will be somewhat too high.

4. When work is done by human limb movements, muscles have to be used to maintain the posture of the body as a whole and to fix in position parts of the limbs that would otherwise be moved by the contraction of the muscles that do work. For example, in bicycle-pedalling muscles must be used to prevent flexion at the ankle joint and with heavy loads to keep the body in the saddle. If it be assumed that the force that must be exerted by the statically contracting muscles is proportional to the external force exerted by the muscles doing work, then it is possible to find for the efficiency an equation that takes account of this factor. From this equation it can be shown: (1) that the efficiency, as in the simpler case, will be independent of the load; (2) that the general form of the efficiency-time curve will be the same; (3) that with slow speeds the falling off of efficiency will be greater than that predicted for the simpler case, and (4) that in fitting a theoretical curve to the experimental results the values of the constants a and b deduced will be less than those obtained by fitting a curve derived from the simpler equation.

5. The effect of bringing fresh muscles into play and changing the type of movement has already been considered in an earlier part of the discussion.

6. The external mechanical conditions under which a muscle works will affect efficiency. When dealing with isolated muscles a mechanical device is employed so that the whole of the external force exerted can be used in doing measured work. When a machine is worked by human limb movements this condition is not necessarily fulfilled. For example, in the case of bicycle-pedalling only the component perpendicular to the pedal crank of the force actually exerted on the pedal is used in

doing external work. In general, therefore, the efficiency found must be less than would be found under more advantageous mechanical conditions.

The way in which the measured efficiency of human muscular movements would differ, as a result of the operation of these factors, from a predicted efficiency based on equation (3) (supposing the values of the constants were known) may be summarised as follows. All the factors except the first will operate so as to make the measured efficiency lower than the predicted efficiency. The general form of the efficiency-time curve will be the same, but the detailed form of the curve will be altered in such a way that the apparent values of the constants a and b found on the assumption that the efficiency is given by equation (3) will be greater than their actual values.

Summary.

1. The relation previously found between the maximum speed of pedalling a bicycle and the force overcome led to the prediction that the mechanical efficiency of bicycle-pedalling would vary with the speed, and that there would be an optimum speed at which the efficiency is highest.

2. Experiments were made to determine the efficiency of bicycle-pedalling over as wide a range of speeds as possible. The efficiency was found to vary with speed in accordance with the prediction. The optimum time of one foot-movement (half a pedal revolution) was found to be 0·9 sec.

3. At a constant speed of 33 complete pedal revolutions per minute it was found that the efficiency was not appreciably affected by change of load within a range of 5 to 26 kg. at the pedal.

4. The agreement of the experimental results with the theoretical efficiency-time curve shows that the chief factors involved in human muscular movement are taken account of in the equation given. It is pointed out, however, that various complicating factors exist which prohibit too full an acceptance of the exact values of the constants deduced.

My thanks are due to Professor A. V. Hill for his interest and help throughout the investigation, to the Medical Research Council for a grant to enable the work to be done, and to Mr J. L. Parkinson for assistance with the experiments.

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THE DISAPPEARANCE OF HISTAMINE FROM AUTOLYSING LUNG TISSUE.

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The presence of histamine in extracts of normal lung accounts for a very large proportion of the vaso-dilator activity of these extracts(1). The physiological significance of the occurrence of histamine in normal tissue has been discussed by Best, Dale, Dudley and Thorpe. The question of changes in the amount of histamine in lung tissue during autolysis has not previously been investigated, and it was thought that interesting information might be obtained from a study of this problem. The effect of autolysis of minced lung tissue on the naturally occurring and on added histamine is recorded in this communication. It has been found that histamine disappears during the autolysis.

Methods.

Autolysis. Perfectly fresh ox or horse lung was finely minced and the minced material was mixed by passing it several times through the mincer. Weighed samples of this material were then treated with hydrochloric acid, as will be described later, to determine the initial histamine content of the lung tissue. 20 grm. portions of the minced lung were then weighed out and transferred to 200 c.c. flasks. 100 c.c. of physiological saline and 15 c.c. of toluene were added to each flask. The flasks were placed in an incubator which was kept at 37° C. The contents of the flasks were well mixed by shaking at hourly intervals for the first five or six hours. Flasks were removed from the incubator from time to time and histamine estimations carried out.

Histamine estimation. The histamine was estimated by the following procedure which has been found by McHenry and the author in the Department of Physiological Hygiene, University of Toronto, to give consistent results and invariably to recover added histamine within the limits of the error of the physiological method used in the comparison of the final solution with a standard solution of histamine. To 20 grm. of tissue, or to a suspension of this amount of tissue in saline, 50 c.c. of

concentrated hydrochloric acid are added. The mixture is heated under a reflux condenser until the liquid boils. The heat is then slightly reduced and the temperature of the liquid kept at approximately 95° C. for 30 minutes. Histamine is not lost when the solution is boiled for a much longer period with larger amounts of hydrochloric acid (Koessler and Hanke(2)). The mixture is then transferred to a round bottom flask and is concentrated *in vacuo* approximately to dryness. The residue in the flask is treated with 200 c.c. of 95 p.c. alcohol, and this is removed by vacuum distillation. Two or three treatments with alcohol are carried out. The residue is then suspended in distilled water and 40 p.c. sodium hydroxide added until the solution is neutral to litmus. The mixture is then filtered. The clear filtrate may contain 2 p.c. sodium chloride or slightly more, but in the small amounts injected this salt does not interfere with the physiological assay. The method of assay of histamine on etherised cats was identical with that used in previous researches from this laboratory. In a fairly sensitive cat it is possible to discriminate between doses of histamine which differ from each other by 15 p.c. The histamine values are expressed in mg. per 100 grm. of tissue. The test dose of histamine was usually approximately 1/1000 mg. As there was usually about 10 mg. of histamine per 100 grm. in the extract at the beginning of the experiment, a slight experimental error in testing is multiplied by a large figure in the calculation of the total amount of histamine present. "Ergamine diphosphate" (Burroughs Wellcome and Co.) has been used as the standard and the weight of the base taken as one-third that of the salt. The standard solution of histamine was usually prepared on each test day. It has been found, however, that a solution of histamine will retain all its depressor activity for at least a month when kept in the incubator at 37° C. or in the laboratory at room temperature if toluene to the extent of 10 p.c. by volume is added and the container is shaken occasionally.

Rate of autolysis. In the first experiment the progress of the autolysis was followed by determining the increase of non-protein nitrogen and of amino-acids in the mixture. These determinations were carried out as follows: 30 c.c. of the minced lung in saline mixture were treated with sufficient trichloroacetic acid to secure a concentration of 2 p.c. The mixture was shaken and allowed to stand overnight. It was then filtered. Total nitrogen was determined in the clear protein-free filtrate by the Kjeldahl procedure. To estimate the amino-acids 10 c.c. of this filtrate were neutralised with 0·1 *N* sodium hydroxide using phenolphthalein as the indicator. Formaldehyde(3) neutralised in an identical

manner was added and the acid liberated was determined by titration with 0·1 *N* sodium hydroxide. Phenol-phthalein was used as the indicator in all three titrations and the same faint pink tint was adopted. Non-protein and amino-acid nitrogen were determined in the first experiment only.

Experimental results.

Natural histamine. In the first experiment two 20 grm. portions of minced horse lung were treated as described above to determine the histamine originally present. The solutions obtained were found to contain vaso-dilator material corresponding to 5·9 mg. of histamine per 100 grm. of original tissue. It was not possible to detect any difference between vaso-dilator effects of equal amounts of the final solutions obtained in the duplicate determinations. Equally satisfactory agreement between duplicates was consistently found throughout this series of experiments. Two flasks each containing 20 grm. of the minced tissue, saline, and toluene were removed from the incubator after 2, 5, 9, 15 and 25 days at 37° C. Samples of the mixture were removed for nitrogen estimations and the vaso-dilator activity of the remaining solution was determined. Fig. 1 shows the disappearance of the vaso-dilator activity

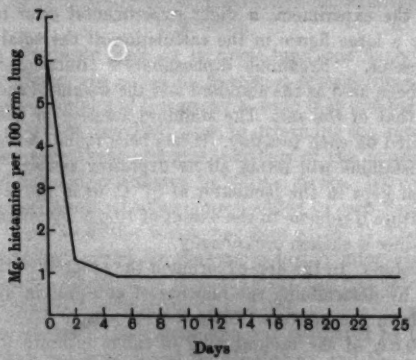

Fig. 1. Natural histamine.

during incubation. The figures show that the histamine content of the mixture is constant within the limit of experimental error after the fifth day of incubation.

In this experiment the non-protein nitrogen was determined at the

beginning, and after 2, 5, 9 and 15 days. The values were as follows: 0·33, 0·54, 0·71, 0·82 and 1·00 grm. per 100 grm. of tissue. The figures for the amino-acid nitrogen showed a similar increase. Although the histamine content of the solution did not change appreciably after the fifth day it will be seen that the non-protein and amino-acid nitrogen continued to increase after that time.

Histamine added. When the two flasks had been removed on the fifteenth day, a second experiment was started. To the contents of each of eleven remaining flasks, which had already been incubated for 15 days, 5 mg. of histamine were added. Histamine determinations were made immediately on the contents of two of the flasks. The value obtained was 5·2 mg. The determination made on similar material before the addition of the 5 mg. of histamine at the fifteenth day indicated 0·16 mg. The recovery of the added histamine was therefore satisfactory. The contents of four of the flasks were heated at 90° C. for 4 minutes. The nine flasks were then returned to the incubator. Determinations made two days later showed 5·25 mg. in the flasks the contents of which had been heated. The unheated samples contained 3·4 mg. Six days after the histamine was added, the heated flasks showed 4·85 and the unheated 1·6 mg. histamine. A determination made on the eighth day on the remaining flask showed 1·7 mg. These two experiments show that both natural and added histamine disappear during autolysis under the conditions of these experiments. Heating at 90° C. for 4 minutes prevents this disappearance.

In a third experiment the histamine value fell from 10 mg. at the beginning to 0·8 mg. per 100 grm. on the ninth day of incubation, 5 mg. of histamine per 100 grm. were present in the tissue and 5 mg. were added.

Figs. 2 and 3 illustrate the results of the fourth and fifth experiments. The lung used in Exp. 4 (Fig. 2) was not quite as fresh as that used in the other experiments. This may explain the slower disappearance of the histamine in the unheated samples in this experiment. In the fifth experiment there is a more rapid disappearance of natural and added histamine. In both these experiments the heated samples show no detectable loss of the vaso-dilator substance.

Reaction of mixture. The *p*H of the liquid in which the minced lung was suspended was approximately 7·2 at the beginning of the experiments. In one experiment in which the solution was adjusted to *p*H 2 with hydrochloric acid, there was no disappearance of histamine during six days' incubation. The same solution at *p*H 7·2 showed a large decrease in histamine content during the same incubation period.

Other organs than lung. In a few preliminary experiments in which tissues other than lung were studied, it was found that minced liver or

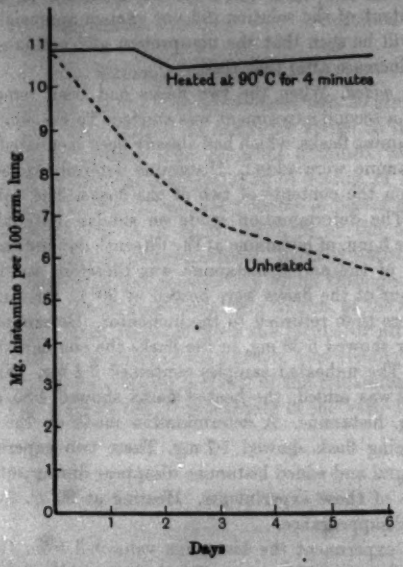

Fig. 2. Natural plus added histamine. 5 mg. natural—6 mg. added.

kidney also possessed the property of causing histamine to disappear. In one experiment no detectable amount of histamine disappeared from a suspension of minced muscle during six days' incubation. In the experiment with liver and kidney, 5 mg. of histamine per 100 grm. of tissue were added. The original samples of liver and kidney contained 0·6 mg. and 0·3 mg. vaso-dilator activity expressed as histamine per 100 grm. At the end of six days' incubation the unheated flasks contained 0·54 mg. and 1·1 mg. per 100 grm. There was no perceptible loss of vaso-dilator activity in the control samples which had been heated at 90° C. for 4 minutes after the addition of the 5 mg. of histamine.

Absence of bacteria. It has been stated by Bradley(4) and by others that microorganisms do not multiply in a suspension of tissue containing 10 p.c. toluene if the mixture is shaken every few hours during

the first 24 hours of incubation at 37° C. I am indebted to Dr P. P. Laidlaw for making cultures from several of the lung suspensions used

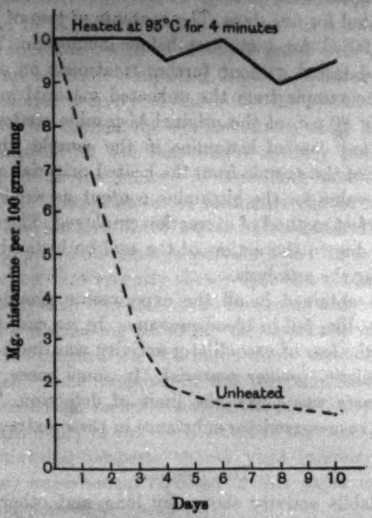

Fig. 3. Natural plus added histamine. 5 mg. natural—5 mg. added.

in these experiments, and for examining stained film-preparations from similar material. The suspensions from which the cultures were taken had not been heated at 90° C. and had been incubated at 37° C. for 15 days. There was no growth in some of the culture tubes, while in others growth was apparent only after 24 or 48 hours. This finding was taken to indicate the absence of rapidly growing vegetative forms of bacteria. Film-preparations were made from samples of three suspensions which had been incubated for 25 days. No organisms were found.

In several experiments the findings of Best, Dale, Dudley and Thorpe that lung contains very little vaso-dilator activity which is eliminated by atropine were confirmed. The administration of atropine did not appreciably change the value for the assay of extracts made from material taken at the beginning or the end of the period of autolysis.

The faint possibility existed that some change might be produced

in the histamine during the autolysis which would render it susceptible to the strong hydrochloric acid used in the preparation of the extracts for testing. Samples were therefore removed from four flasks which had been incubated for five days. The contents of two of the flasks had been heated at 90° C. for 4 minutes before incubation. These samples were filtered and tested without further treatment on etherized cats. The assay on the sample from the unheated material indicated a loss of approximately 80 p.c. of the original histamine content. There was no evidence of any loss of histamine in the sample which had been heated. The test of the sample from the heated material gave only such a slightly lower value for the histamine content as would be expected with the less perfect method of extraction employed. The disappearance is therefore not due to the action of the acid on histamine made more susceptible during the autolysis.

The extracts obtained in all the experiments produced a characteristic histamine-like fall in blood-pressure. In no case was there any suggestion that the loss of vaso-dilator activity was due to the presence of some antagonistic, pressor material. In some cases the histamine content of extracts was below the limit of detection. There was no evidence of any vaso-constrictor substance in these extracts.

Discussion.

The thermolabile activity shown by lung and other tissues incubated in a saline suspension, which causes the disappearance of naturally occurring or added histamine, may prove to possess physiological significance. The experiments recorded in this paper are not concerned with a possible vital function of this action, but only with the phenomenon as seen and studied *in vitro*.

The findings, (*a*) that the disappearance of the histamine is completely prevented by heating the suspension at 90° C. for 4 minutes; (*b*) that the disappearance proceeds rapidly during the first period, becomes slower, and practically ceases while there is still histamine available; and (*c*) that the addition of more histamine to this mixture produces a renewed disappearance of the vaso-dilator substance, all indicate that the property of lung here investigated possesses some of the characteristics of an enzyme.

The experiments in which atropine was administered show that choline is not an important factor in these results. There is no suggestion that the presence of histamine is masked by the production of vaso-constrictor substances. The time relationships of the action of the known

vaso-constrictor materials are such that the sharp fall in blood-pressure and the return to the normal level produced by histamine would be changed if appreciable amounts of these substances were present. It has been shown by Kendall(5) that histamine is inactivated by formaldehyde. If a substance, with an aldehyde group were produced during the autolysis, an addition compound might be formed with histamine and the vaso-dilator activity of the latter destroyed. This compound would probably be broken up by the strong acid, but whether the structure responsible for the vaso-dilator activity would then be left undisturbed is not as yet known. A more likely possibility is that the mechanism of the disappearance of histamine will be found to be oxidative, since Ewins and Laidlaw(6) have shown that tyramine is oxidized during perfusion through isolated liver. In a very interesting paper Miss Hare(7) has recently shown that this oxidation is due to a definite enzyme system. The fate of the histamine which has disappeared presents a very fascinating problem, the approach to which will be facilitated by the preparation of a much more potent histamine-inactivating material. Some progress in this direction has been made in experiments carried out recently with E. W. McHenry in Toronto.

The thorough study of the distribution in the various tissues of this histamine-inactivating property should yield interesting information. The preliminary experiments reported in this paper demonstrate that kidney and liver possess this property.

Summary.

It has been shown that lung and other tissues, when suspended in saline and incubated in the presence of toluene at 37° C., cause the disappearance of naturally occurring or added histamine. The substance or system producing this disappearance is thermolabile.

It is a pleasure to express my deep appreciation to Dr H. H. Dale for the hospitality of his laboratory and for his active and helpful interest in this work. I am also indebted to Dr H. W. Dudley for help in many ways.

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THE ACTION OF EXTRACT OF PITUITARY ON THE BLOOD SUGAR AFTER PANCREATECTOMY.

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The experiments about to be described were undertaken in order to observe the action of pituitary extract on the blood sugar in an animal deprived of its pancreas and maintained in a healthy condition on an appropriate diet and adequate doses of insulin. Under such conditions the amount of insulin and glycogen in the tissues could be more or less controlled and the influence of these factors observed. The investigation was suggested by the observations that pituitary extract[1] diminishes the hyperglycæmia in diabetes mellitus (1) and causes a fall in the blood sugar of eviscerated cats receiving glucose injected intravenously at a uniform rate (2). These effects are not understood. Clark (2) refers to the observations of Ahlgren (3), who found that pituitary extract increases the oxidation processes in muscle tissue and suggests this as a possible explanation of the fall in the blood sugar produced by the extract in his eviscerated preparations. It was thought that some information regarding the nature of these responses might be furnished by a study of the effects of pituitary extract on the hyperglycæmia of depancreatized animals.

Such preparations would also provide an opportunity of investigating the source of the additional glucose in "pituitrin" hyperglycæmia. Clark's observations suggest that it comes from the glycogen of the liver. They do not support the view of Moehlig and Ainslie (4) that the extra glucose arises from the glycogen in the muscles. Since MacLeod and his co-workers (5) were unable to detect any significant difference in the amount of glycogen present in the skeletal muscles of depancreatized animals before and after insulin, but observed an increase in the liver, it was thought that by withholding insulin for varying periods of time and depleting the liver of its glycogen, additional evidence regarding the source of the glucose in "pituitrin" hyperglycæmia might be obtained.

[1] The pituitary extract employed was the preparation issued by Park, Davis and Co., under the name "pituitrin."

Methods. A fox terrier bitch, weight 14 lb., was depancreatized under ether anæsthesia. For the first 48 hours water, cane sugar and insulin were given. It was then placed on the following diet: meat 400 grm., raw pancreas 100 grm. and cane sugar 100 grm. (MacLeod(5)). This was divided into two feeds given morning and evening. Fourteen units of insulin were injected twice daily after the sugar had been taken. During the first few days the animal ate only part of the diet so that the dose of insulin was less, but by the end of a week the full diet was being consumed. Under this regime the dog survived for 10 months in an excellent state of health, the body weight was maintained and the skin remained in good condition.

In all the experiments blood was obtained from the ear at half-hourly intervals and the sugar estimated by the method of Hagedorn and Jensen(6). The observations were made in the afternoons, and no food or insulin was given on the mornings of such days except when stated otherwise. During the experiments the animal was kept in a box devised for the purpose and curtained off in a corner of the laboratory. This procedure was found to be desirable as at times emotional stimulation appeared to influence the shape of the blood sugar curves.

Experiments. Blood sugar curves after the injection of 1 c.c. of "pituitrin" were first obtained on the intact animal before the pancreas was removed. Three of these are set out in Table I. The fasting level of the blood sugar is about 0·095 p.c. and the response to "pituitrin" is similar to that previously observed for this and other animals. In the third series the return of the blood sugar to normal is delayed, but in this instance the animal had received food 5 hours before.

TABLE I.

Blood sugar after 1 c.c. of "pituitrin" in normal dog, intervals of 30 minutes between determinations.

A. (p.c.)	B. (p.c.)	C. (p.c.)
0·093	0·093	0·102
0·090*	0·097*	0·107*
0·150	0·130	0·146
0·083	0·103	0·134
0·097	0·102	0·122
0·102	0·093	0·109
0·100		

* "Pituitrin" was given immediately after this sample of blood was withdrawn.

Observations on the depancreatized animal were begun a fortnight after the operation. By this time the wound had healed, the original weight was regained and the dog accustomed to its diet and injections

of insulin. The blood sugar was determined over periods of 2 to 3 hours under different conditions as regards food and insulin. In one instance food and insulin were given in the morning and observations begun 5 hours later, in others no morning ration of food or insulin was given, and in others, again, insulin was withheld for periods varying from 20 to 95 hours before the examinations were made. During the longer periods of insulin deprivation the animal lost weight and on one occasion became quite ill. Several days intervened, therefore, between such observations to enable the former state of nutrition to be regained. The figures obtained on four occasions are given in Table II.

TABLE II.

Food and insulin at 9.30 a.m. (p.c.)	No food or insulin in the morning (p.c.)	No food in morning, no insulin for 44 hours (p.c.)	No food in morning, no insulin for 70 hours (p.c.)
0·167	0·206	0·242	0·160
0·181	0·201	0·204	0·158
0·201	0·224	0·200	0·163
0·200	0·218	0·183	0·158
0·237	0·208	0·179	—
—	—	0·161	—

It is apparent that wide variations in the blood sugar occurred when insulin had been withheld for 44 hours or less; but after 70 hours of insulin deprivation the blood sugar remained constant for 2 hours, though at a lower level. If glycogen were present in the liver in those instances when insulin had not been withheld longer than 44 hours it is likely that variations in the blood sugar were associated with the liberation of glucose from it, for the animal was restless, excitable and barking at times and in a state where emotional stimulation must have been a factor. If no glycogen were present in the liver after 70 hours of insulin deprivation the emotional state of the animal should not have influenced the blood sugar. It did not do so in the instance referred to or on eight other occasions which will be mentioned later. The injection of 0·3 c.c. of adrenaline (1 in 1000) did not produce a rise in the blood sugar when insulin had not been given for 70 hours, indicating the absence or low concentration of glycogen in the liver. When adrenaline was given after insulin had been withheld for only 20 hours a rise occurred. The figures are given in Table III.

The action of pituitary extract was next observed after varying periods of insulin deprivation. One c.c. of "pituitrin" was injected on each occasion. The results are set out in Table IV.

TABLE III.

No insulin for 20 hours (p.c.)	No insulin for 70 hours (p.c.)	No insulin for 70 hours (p.c.)	Hæmoglobin determinations (p.c.)
0·206	0·164	0·181	97
0·198	0·170	0·181	97
0·172*	—*	0·177*	96
0·214	0·165	0·185	102
0·195	0·170	0·184	98
0·175	0·168	0·184	98
—	—	0·187	97

* Adrenaline was given immediately after this sample was withdrawn.

TABLE IV.

20 hours (p.c.)	44 hours (p.c.)	70 hours (p.c.)	70 hours (p.c.)	70 hours (p.c.)	Hb (p.c.)	94 hours (p.c.)
		No insulin given for:				
0·198	—	—	—	—	—	0·171
0·209	0·204	0·174	0·171	0·167	—	0·175
0·202	0·207	0·176	0·171	0·166	100	0·177
0·212*	—*	0·172*	—*	0·168*	100	0·177*
0·245	0·259	0·169	0·171	0·168	100	0·162
0·209	0·170	0·168	0·178	0·165	97	0·161
0·219	0·199	0·154	0·159	0·154	98	0·164
0·209	0·210	0·168	0·180	0·162	98	0·156

* 1 c.c. "pituitrin" given immediately after this sample was withdrawn.

As these figures show, "pituitrin" produced a rise in the blood sugar when insulin had been withheld for 20 and 44 hours. Since considerable variations occurred spontaneously under similar conditions but with no "pituitrin," these results cannot be stressed. In those instances where no insulin had been given for 70 hours or more, no rise followed the injection of "pituitrin," the blood sugar remained remarkably constant except for a slight fall which appeared 1½ to 2 hours later. This fall is comparable in time and degree to that which sometimes occurs in normal animals and man. No fall similar to that observed in eviscerated cats and in diabetes mellitus was obtained. On one occasion hæmoglobin determinations by the Gower-Haldane method were made in order to ascertain whether dilution of the blood could account for the late fall in the blood sugar. No evidence for this was obtained. Similar determination of the hæmoglobin after adrenaline (Table III) in one instance showed a small increase in the concentration of the blood half an hour after the adrenaline was given. This change was not reflected in the blood sugar.

It appears, therefore, that in depancreatized animals "pituitrin" causes a rise in the blood sugar so long as there is glycogen present in

the liver. When insulin is withheld for about 70 hours the hyperglycæmic effects of "pituitrin" are no longer apparent. Collip(7) found that if large amounts of sugar were given with insulin, the liver contained 20 p.c. or more of glycogen. Liberal amounts of sugar were given to this dog, the liver presumably contained much glycogen and it required about 3 days without insulin to remove it. Since the muscles still contained glycogen, these results confirm the view that the glycogen of the liver is the source of the extra glucose in the hyperglycæmia caused by extract of pituitary.

Note. The dog lived for 10 months after the operation. It appeared to be in excellent health except during the longer periods of insulin deprivation, when it lost weight and became less lively. During the last month of its life it refused to take the raw pancreas. At the end of the month it became acutely ill, refused all food and died in 2 days. MacLeod(5) found that without raw pancreas in the diet his animals did not as a rule live longer than 6 weeks and died with symptoms of hepatic insufficiency, and at autopsy a remarkable degree of necrosis and fatty change was found in the liver. The liver in this animal showed the changes described by MacLeod, and in addition the kidneys presented extensive fatty changes most marked in the convoluted tubules but quite abundant in the straight and collecting ones as well. No pancreatic tissue was found.

Summary.

The action of pituitary extract on the blood sugar of a depancreatized animal maintained in a healthy state on an appropriate diet and adequate doses of insulin has been studied.

If insulin is withheld for 44 hours or less before injecting the extract of pituitary, a rise in the blood sugar is produced as in the intact animal.

If insulin is withheld for 70 hours or more, the rise does not occur. A slight fall appears about 1½ to 2 hours after the injection. Under similar conditions adrenaline too fails to increase the concentration of the blood sugar.

Since the muscle glycogen is not materially altered under the conditions of the experiment, these results support the view that the extra glucose in the hyperglycæmia produced by pituitary extract is derived from the glycogen in the liver.

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FATIGUE OF THE SENSE ORGANS IN MUSCLE.

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In view of modern ideas regarding the mechanism of vital processes it seems inevitable that all living tissue should tend to show a progressive decrease in capacity for response as a result of previous activity. This general property we may describe as fatigue. Although the inclusiveness of the designation must indicate that many of the phenomena which may properly be included thereunder are in many respects dissimilar, they are all alike in that the altered condition is in some way a result of the increased metabolism resulting from previous response to stimulation. Because of this the study of fatigue processes in normal and excised tissue often throws light on the dynamics of the cell or organ and on the functional mechanisms involved. The present investigation is therefore devoted to a study of the action of peripheral sense organs during and after a prolonged response to a continuous stimulus. So far as I am aware there has never been such an objective study of the fatigue of sensory endings, and it is only now made possible by the methods which Adrian(1) has developed for the study of sensory impulses.

Evidence is existent of the fatigue of other portions of the nervous system. The work of Sherrington(2), for instance, has demonstrated that there is a fairly rapid fatigue of that portion of a reflex arc lying within the spinal cord, and the common statement regarding the infatigability of nerve fibres has been discredited by recent work, including that of Gerard, Hill and Zotterman(3) and Gerard(4).

The sensory endings in muscle were chosen as being best suited to an investigation such as this. They are easily stimulated with stimuli of known intensity, reproducible results are readily obtained and one can choose a muscle with few enough end organs to make possible the counting of the individual sensory impulses even at the height of the discharge. The preparation employed was the muscle *flexor superficialis digitorum* with the nerve *tibialis superficialis*. The muscle is large enough

to withstand considerable tension and contains a sufficient number of end organs to enable one to observe the way in which fatigue develops within such a group. When it was desired to study the action of a single or a very few endings a majority of the sensory nerve fibres was transected by the method recently described (5). Fig. 1 *A* shows the discharge

Fig. 1. Discharge of afferent impulses from all of the functioning sense organs in the muscle (*A*) and from single organs (*B* and *C*). In *B* and *C* the nerves had been partially transected so that in each case there was only a single sensory fibre conducting impulses from one end organ. White marks give time in 1/8 sec.

from all of the afferent endings in a muscle; 1 *B* and *C* the discharges from apparently single end organs, all but one of the active sensory fibres having been cut between the muscle and the electrodes.

All of the experiments were made on non-circulated, excised tissue. The muscle was clamped in one compartment of a double moist chamber and a thread from the tendon was attached to a loading lever, this lever being lowered at a definite rate by means of an oil dashpot. The nerve passed into the second compartment where it was slung across two non-polarizable electrodes which in turn connected to a 3-valve amplifier and a capillary electrometer.

Fatigue of response to continuous tension.

When a load is applied to a muscle the frequency of afferent impulses rises rapidly to a maximum and then within a few seconds falls to a frequency from which the decrease is much more gradual. This rapid fall in impulse frequency following the rise to maximum has been ascribed

by Adrian and Zotterman(6) to a decline in the effectiveness of the stimulus, i.e. to an adaptation of the end organ to the change in its environment. Although the question whether this initial "adaptation" can be properly classed as a fatigue process is largely a matter of terminology, and although it is doubtful whether this initial fall can be clearly distinguished from the true fatigue which occurs with prolonged loading, it is certain that the onset of adaptation is very rapid and the recovery therefrom equally so. Adrian and Zotterman give figures to show that recovery is practically complete within a second. Several experiments made in the course of the present investigation show that if tension is applied to a muscle just long enough to permit the afferent discharge to fall to its adaptation level, then released and applied again within one or two seconds, the stimuli may be repeated several hundred or even thousand times without causing any large decrease in response. This is illustrated in Fig. 2. It constitutes further evidence for rapid

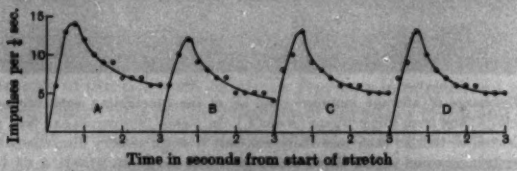

Fig. 2. Effect of repeated stretches on discharge of afferent impulses. 13·6 grm. *A*, 1st stretch; *B*, 102nd stretch; *C*, 163rd stretch; *D*, 250th stretch. About 1-sec. intervals between the end of one loading and the start of the next. Expt. of 10. x. 28.

recovery from the initial fall which Adrian and Zotterman regard as adaptation to the stimulus.

The frequency of afferent impulses throughout the early part of a discharge is higher as the result of a greater stretching force provided this stimulus does not become supra-maximal. Both the maximum impulse frequency and the "adapted frequency" are higher as a result of the greater stimulus. That this increased activity does not continue however under a prolonged tension is shown by Fig. 3 which gives the results of an experiment continued over 24 min. It will be seen that a tension of 45 grm. caused an eventual fall in frequency of afferent impulses to a lower value than that resulting from the smaller stimulus. When the tension was suddenly reduced from 45 grm. to 4 grm. the frequency gradually rose to a new level. The obvious interpretation of the first observation is that the greater rate of activity of the end organs

in response to the greater stimulus induces more rapid and more complete fatigue. It brings the action of these sensory endings into line with

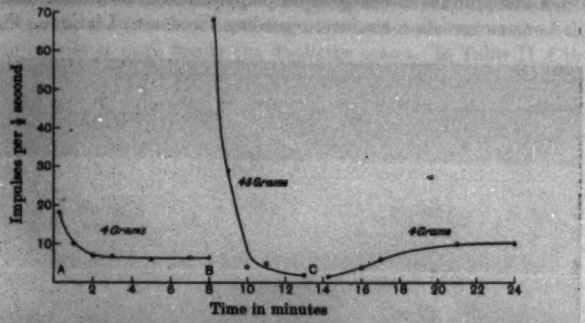

Fig. 3. Effect of varying sustained tension on fatigue. At *A* muscle loaded with 4 grm. At *B*, 41 grm. was added and at *C* the tension was again reduced to 4 grm. Expt. of 28. v. 28.

other excitable tissue in this respect. For instance, a muscle stimulated at a high frequency will soon fatigue, whereas at a lower frequency it may continue contracting indefinitely in a steady state. It is more difficult to find an analogy for the partial recovery from such fatigue during the response to a weaker stimulus. That the increase in frequency under the smaller tension is actually a recovery process, although but partial, is shown by Table I: it will be noted that on again increasing

Table I. Fatigue due to sustained tension.

	Impulses per ½ sec.
15 sec. after loading with 45 grm.	160
1 min. „ „ „	110
2 min. „ „ „	48
3 min. „ „ „	3
7 min. „ „ „	2
15 sec. after reducing tension to 4 grm.	3
5 min. „ „ „ „	7
10 min. „ „ „ „	12
15 sec. after again increasing tension to 45 grm.	74
1 min. „ „ „ „ „	24
2 min. „ „ „ „ „	3
7 min. „ „ „ „ „	3
15 sec. after tension was again reduced to 4 grm.	1
2 min. „ „ „ „ „	3
6 min. „ „ „ „ „	9

the tension the frequency rises and then falls more quickly than it did when this same load was first applied.

A study of the discharge from preparations containing a number of end organs reveals some facts regarding the onset of fatigue. Fig. 4 *A*

Fig. 4. Electrometer records of the sensory discharge from a muscle under tension of 45 grm. showing the progressive fatigue of end organs. *A*, 30 sec. after the application of the load, discharge from three or more sensory endings. *B*, after 2 min., only one organ active; and *C* after 3 min., no response. White marks give time in 1/8 sec. Expt. of 24. v. 28.

shows the impulse discharge 30 sec. after the initial application of the tension; *B*, 2 min. later. Comparing these two photographs with Fig. 1 *A* and *B* it will be seen that the discharge early in the loading is of the type resulting from the activity of a fair number of end organs, while after a period of sustained tension there is a definitely regular rhythm characteristic of the discharge from a single sensory ending. In Fig. 4 *C* the discharge has entirely disappeared 3 min. after the start of the loading. The natural conclusion to draw from these experiments is that the development of fatigue is marked by more and more end organs dropping out of action due to progressive failure.

This is not to be taken as indicating that the individual sense organ responds at a characteristic frequency until no longer able to do so and then abruptly ceases to function. Adrian and Zotterman have shown that a single end organ is able to discharge over a considerable range of frequencies, and this same point has been established in the present work. In some preparations the decrease in response as the loading continues has been marked by a gradual and progressive fall

in the rhythmic impulse frequency. More frequently, and especially after previous fatigue of the end organ, the failure of response is characterized by one or more impulses dropping out of an otherwise fairly regular series, the impulses becoming more and more scattered as the loading continues until finally the discharge ceases. In Table II *A* the

TABLE II. Intervals between successive impulses coming from a stretched muscle.

The series start from the beginning of the stretches. The intervals are expressed in terms of arbitrary time units (1/40 sec.). Series *A* and *B* are both from the same muscle, *B* from a later part of the experiment.

A. 3·7, 2·2, 2·1, 1·9, 1·6, 1·2, 1·1, 1·0, 0·9, 0·9, 0·9, 0·9, 0·9, 0·9, 0·9, 0·9, 0·9, 0·9, 0·9, 0·9, 0·9, 0·9, 0·9, 1·0, 1·0, 1·0, 1·0, 1·0, 1·0, 1·0, 1·2, 1·2, 1·2, 1·2, 1·2, 1·2, 1·2, 1·2, 1·3, 1·3, 1·3, 1·3, 1·3, 1·3, 1·3, 1·3, 1·3, 1·3, 1·5, 1·8, 1·7, 1·8, 1·8, 1·8, 1·8, 1·8, 1·8, 1·8, 1·9, 1·9, 1·8, 1·8, 1·9, 1·9, 1·9, 1·7, 1·9, 1·7, 2·0, 1·8, 1·8, 2·0, 2·0, 2·0, 2·0, 2·2, 2·2, 2·0, 2·2, 2·1, 1·7, 2·2, 1·8, 2·0, 2·0, 2·0, 2·1, 2·3, 2·3, 2·3, 2·3, 2·7, 2·6, 2·9, 2·8, 2·0, 2·8, 2·6, 2·7, 2·6, 2·8, 2·7, 2·7, 2·8, 2·9, 2·8, 2·8, 3·2, 3·2, 3·2, 2·9, 3·0, 3·0, 2·9, 3·0, 3·1, 3·1, 3·0, 3·2, 3·1.

B. 2·0, 1·9, 1·9, 1·3, 1·1, 1·0, 0·8, 0·8, 0·8, 0·7, 0·7, 0·7, 0·6, 0·6, 0·6, 0·6, 0·6, 0·6, 0·6, 0·7, 0·7, 0·6, 0·6, 0·7, 0·6, 0·6, 0·5, 0·5, 0·5, 0·5, 0·5, 0·5, 0·5, 0·5, 0·5, 0·5, 0·5, 0·5, 0·5, 0·5, 0·6, 0·5, 0·6, 0·6, 0·6, 0·6, 0·7, 0·7, 0·7, 0·7, 0·7, 0·7, 0·7, 0·7, 0·7, 0·7, 0·7, 0·7, 0·7, 0·7, 0·7, 0·7, 0·8, 0·8, 0·8, 0·8, 0·7, 0·8, 0·8, 0·8, 0·9, 0·8, 0·8, 0·8, 0·8, 0·9, 0·9, 0·9, 0·9, 0·8, 0·8, 0·8, 0·8, 0·8, 0·8, 1·3, 0·8, 0·8, 0·8, 0·8, 0·8, 0·8, 0·8, 0·8, 0·8, 0·9, 0·8, 0·7, 1·3, 0·8, 0·8, 0·8, 0·9, 2·8, 4·0, 0·8, 0·8, 0·9, 0·8, 9·0, 0·8, 1·4, 0·9, 0·8, 0·8, 8·0, 0·9, 1·5, 3·1, 5·2, 3·1, 5·2, 1·9, 3·0, 1·2.

decline in frequency is fairly regular, the intervals between successive impulses becoming gradually longer; in *B* there is at first a similar regular increase in interval between impulses, but later whole groups of impulses drop out of the series. It does not seem possible at present to say what factors are responsible for these differences in behaviour.

FATIGUE OF RESPONSE TO STRETCH.

Fig. 2 shows that although the frequency of afferent impulses from a muscle quickly falls during the early part of a short loading, such a stimulus has little effect on the response to a subsequent stretch following at an interval of 1 sec. or less. The experiments described in this section relate to the effects of much longer periods of loading upon the discharge of sensory impulses produced by a subsequent stretch following after a brief interval of rest.

The results of a typical experiment are illustrated in Fig. 5. In order to determine the normal response of the muscle to the given stimulus it was loaded several times at 5-min. intervals. These impulse discharges were all in close agreement: one of them is given in *A*. The last of these preliminary stretches was prolonged for a period of 1 min., the weight was then lifted and again lowered after an interval of 15 sec. The early part of the response is given in *B*. The muscle was similarly stretched

after additional periods of sustained tension of 1 min., 3 min., 8 min., and then after "rest" periods of 1, 2, 5, 5, 10 (total of 23) min. respectively interspersed with 30-sec. loadings that produced the afferent

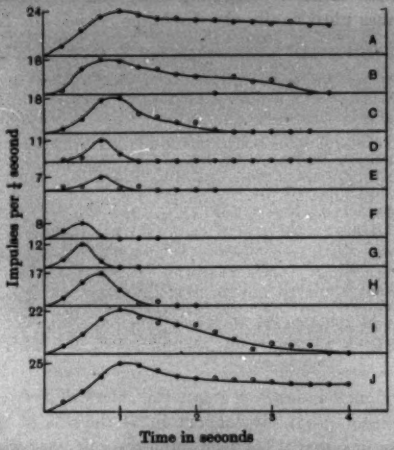

Fig. 5. The discharge of sensory impulses produced by stretching a muscle slowly with a weight of 9·6 grm. *A*, rested muscle; *B*, after a previous continued loading of 1 min.; *C*, 2 min.; *D*, 5 min.; *E*, 13 min. The muscle was loaded another 10 min. and then allowed 1 min. rest before *F*, 3 min. before *G*, 8 min. before *H*, 13 min. before *I* and 23 min. before *J*. The period of tension was interrupted by 15 sec. periods of rest before each of the four loadings; the period of rest by four 30-sec. stretches. Expt. of 9. x. 28.

impulse discharges given in *F*, *G*, etc. The results of this series of observations are presented in a different form in Fig. 6 which gives the degree of the fatigue as a function of the duration of the previous tension. A large number of these experiments have conclusively shown that prolonged tension reduces the capacity of an end organ to respond to a subsequent stretch. The gradual and complete recovery of normal activity makes it seem probable that we are here dealing with a true fatigue phenomenon.

The rate of development of fatigue and recovery is naturally dependent on various conditions such as the state of the muscle, the amount of tension and the like: the values given by the experiments here quoted merely serve to illustrate the general order of magnitudes. The two curves

in Fig. 7, for instance, show the effect of varying tension on the rate of fatigue. As would be expected, the end organs fail more rapidly under a higher tension and recover more slowly from the more complete fatigue.

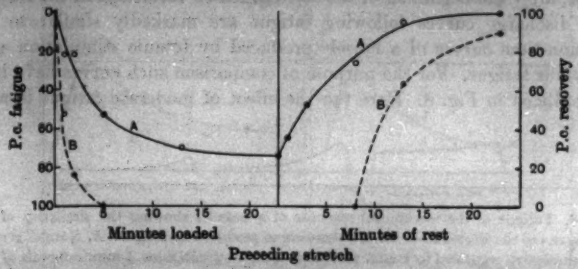

Fig. 6. Rate of fatigue and recovery. *A*. Full line. The maximum impulse frequency in the several stretches is given as per cent. of the maximum frequency in a like stretch of the unfatigued preparation and is plotted against the duration of the previous sustained tension. *B*. Broken line. The impulse frequencies 2 sec. after the start of each stretch are similarly compared during the course of fatigue and recovery. Taken from the series given in Fig. 5.

Following fatigue the discharge frequency during the course of a stretch decreases very much more rapidly than in a normal preparation; the later or sustained discharge may be entirely absent although the

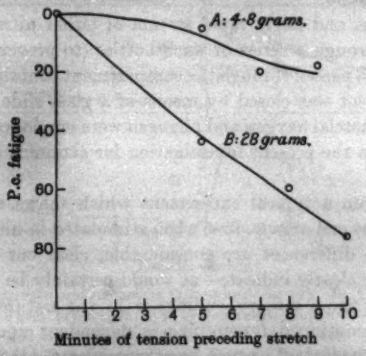

Fig. 7. Rate of fatigue as a function of the load. Maximum impulse frequency during a stretch as per cent. of that from the rested muscle plotted against the duration of a previous tension of (*A*) 4·8 grm., (*B*) 28 grm. Expt. of 25. x. 28.

initial discharge is still of considerable size. This is shown in Fig. 5 *D*, *E*, *F*, *G*, *H*. Fig. 6 illustrates the same point by showing the much greater rate of fatigue obtained when one considers the impulse frequency 2 sec. after the beginning of the stretch. These variations in the form of the discharge curves following fatigue are markedly similar to the tension-time curves of a muscle produced by tetanic stimulation after previous fatigue. For the purpose of comparison such curves have been reproduced in Fig. 8. Here too the effect of moderate fatigue is most

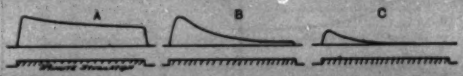

Fig. 8. Fatigue of the mechanical response of a muscle, showing the similarity of this series to the altered response of a sense organ produced by fatigue. *A*, *B* and *C* give the successive responses to 1-min. periods of tetanic stimulation. 1-min. intervals of rest.

evident in the later part of the response, the tension falling more rapidly in the case of the fatigued muscle.

Effects of nitrogen and oxygen.

If the phenomena described above are due to a fatigue of processes similar to those which are characteristic of most living tissues, the rate of fatigue and recovery therefrom should be modified by placing the muscle in an atmosphere of nitrogen or oxygen. This has been done with striking results.

A continuous and fairly rapid stream of either nitrogen or oxygen was bubbled through a series of wash bottles (to prevent drying of the muscle) and then passed through the compartment containing the muscle. This compartment was closed by means of a glass slide sealed on with vaseline. Commercial oxygen and nitrogen were employed: there seemed to be no need in the present investigation for attaining a higher degree of purity.

Fig. 9 is from a typical experiment which shows the fatigue and recovery of these end organs, first when stimulated in nitrogen and later in oxygen. The differences are considerable, clear cut and need little comment. They clearly indicate—as would certainly be expected—that the activity of these sensory endings is in the nature of a chemical process. Their continued functioning is dependent upon an adequate supply of oxygen: lack of oxygen greatly accelerates the fatigue process and retards recovery.

It has been pointed out that the rapid decline in impulse frequency

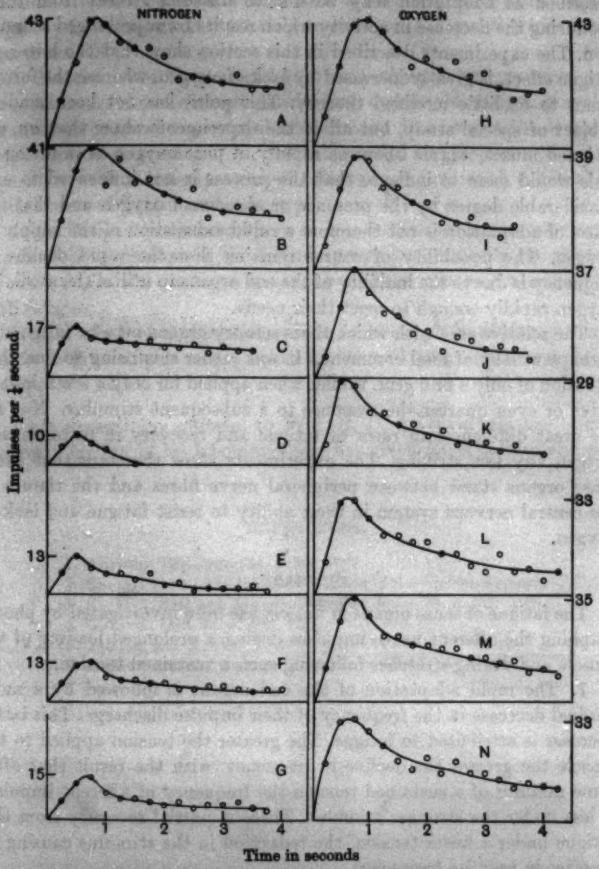

Fig. 9. Effect of nitrogen and oxygen on fatigue and recovery. In *A* to *G* the muscle was loaded with 5·6 grm. in nitrogen; in *H* to *N* the same loads were applied to the muscle in oxygen. *A* and *H* rested preparation; *B* and *I* after 1 min. of a sustained tension of 5·6 grm.; *C* and *J* after 3 min.; *D* and *K* after 7 min. of sustained tension; *E* and *L* after 2 min. rest; *F* and *M* after 6½ min. rest; *G* and *N* after 9½ min. rest. Same muscle employed in both series.

described as adaptation may be due to different causes from those producing the decrease in activity which results from prolonged stimulation. The experiments described in this section show that the latter, or fatigue effect, is greatly increased by lack of oxygen, whereas the former seems to be little modified thereby. This point has not been made a subject of special study, but all of the experiments show that an unfatigued muscle adapts about as rapidly in pure oxygen as in nitrogen. This would seem to indicate that the process is not influenced to any considerable degree by the presence or absence of oxygen and that the cause of adaptation is not therefore a rapid exhaustion of the supply of oxygen. The possibility of course remains that the rapid decline in frequency is due to the inability of the end organs to utilize the available oxygen rapidly enough to meet their needs.

The relative ease with which these sensory organs may be fatigued is perhaps worthy of final comment. It was rather surprising to find that a tension of only 4 or 5 grm. would, when applied for only a few minutes, halve or even quarter the response to a subsequent stimulus. Nor are the great differences in rates of fatigue and recovery in nitrogen and oxygen any less striking. The experiments show therefore that these sense organs stand between peripheral nerve fibres and the tissues of the central nervous system in their ability to resist fatigue and lack of oxygen.

Summary.

The fatigue of sense organs in muscle has been investigated by photographing the afferent nerve impulses during a prolonged loading of the muscle and during stretches following such a sustained tension.

1. The rapid adaptation of the end organs is followed by a more gradual decrease in the frequency of their impulse discharge. This latter decrease is attributed to fatigue. The greater the tension applied to the muscle the greater the decline in frequency, with the result that after some minutes of a sustained tension the frequency of afferent impulses is less under the stronger stimulus. There is partial recovery from this fatigue under a lower tension, the reduction in the stimulus causing an increase in impulse frequency.

2. The decrease in frequency during a prolonged stimulus is due, in part, to the fact that progressively fewer end organs discharge as the stimulus continues. An investigation of single end organs shows that as they fatigue the frequency of their discharge falls. This decrease in discharge frequency in some preparations results from a gradual and

continuous transition from a high to a low value. More often, as the frequency falls, more and more impulses drop out of an otherwise fairly regular series.

3. Muscles have been stretched over a thousand times with only 1-sec. intervals of rest without showing any appreciable fatigue provided the successive stretches were of short duration. If, however, the duration of the tension is prolonged for several minutes the response to a subsequent stretch is greatly reduced. Curves are presented which show typical rates of fatigue and recovery.

4. As fatigue progresses there is less reduction in the maximum impulse frequency than in the frequency at a later stage in the stretch. In other words, the discharge frequency declines more and more rapidly with fatigue.

5. The effect of loading a muscle in an atmosphere of nitrogen is to hasten fatigue of the sense organs and retard recovery. Oxygen has the opposite effect.

I am exceedingly grateful to Prof. E. D. Adrian. He has offered me every facility of his laboratory and has given much helpful advice.

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ADSORPTION OF HÆMOGLOBIN BY MUSCLE TISSUE.

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Introduction.

In a research on muscle hæmoglobin it was found that minced muscle tissue can be completely decolorized by washing it with Ringer's fluid. The same result can be obtained by using 1 p.c. NaCl solution. After removing the salt with distilled water, the tissue colours itself intensely red if shaken up in a test-tube containing water to which a little blood has been added. The colour now obtained can again be removed with 1 p.c. NaCl or Ringer's fluid, but not with water. In this paper some of the conditions under which this colouring and decolorization takes place will be defined.

Methods.

(*a*) *Preparation of muscle tissue.* All experiments were done on ox-muscle, which was minced in a meat-mincer; in earlier experiments it was frozen and finely cut with a microtome; when merely cut up with a pair of scissors the muscle pulp obtained was not uniformly fine enough. To decolorize the pulp 1 grm. was introduced into each of a set of centrifuge-tubes, to which was added 15 c.c. of a 1 p.c. NaCl solution; the tubes were shaken for a short time and then centrifuged. The salt solution was removed after that and the sediment shaken up and centrifuged with distilled water, three times in succession. The absence of salts in the tissue thus treated was controlled by treating the third portion of washing-fluid with $AgNO_3$ or by determining the conductivity. Tissue treated in this way was colourless and was used for the experiments to be described.

(*b*) *Preparation of hæmoglobin.* After preliminary experiments with blood it was decided to eliminate possible complications by using Hb-solutions. The standard Hb-solution was prepared according to the ether method (Adair, Barcroft and Bock(1); Adair(2); Stoddard and Adair(3)) with a few modifications taken from Ferry(4) and Heidelberger(5).

Defibrinated ox-blood was centrifuged and washed five or six times with 1–1½ p.c. NaCl solution, until the washing-fluid showed no albumin reaction on boiling. Before the addition of ether, the blood corpuscles were washed twice with 4 p.c. NaCl solution, so as to cause shrinkage and better settlement. Hæmolysis, which may now take place, is of minor importance, because one has made sure by the boiling test that the serum albumin has been removed. After washing with ether, centrifuging and pipetting off the ether and the layer of stromata, the solution was dialysed in parchment bags. These bags were open as it was not important to obtain a concentrated Hb-solution. When the resistance to an electric current amounted to fully 7000 (as compared with 136 for *N*/10 KCl) the solution was taken from the bags and kept on ice.

The amount of Hb read off on a Bürker colorimeter (6) was found to be 22·8 grm. Hb per 100 c.c. solution; calculated from the dry weight, as done by Stoddard and Adair (3), it was 23·9 grm. Hb per 100 c.c. solution.

(*c*) *Examination of the fluids used for imparting or removing pigment.* The concentration of Hb in these fluids was estimated by means of the comparator spectroscope of C. Pulfrich (7), using a Hb-solution of known concentration, usually 1/500 of the original Hb-solution, as standard.

Results.

(*a*) *Influence of time on depth of colour.* First of all a few experiments were performed to determine how far the intensity of colouring depended upon the time of contact between Hb-solution and tissue. Decolorized and washed tissue (1 grm.) was introduced into each of a series of centrifuge-tubes. These were then shaken for different periods with 10 c.c. of a Hb-solution of known concentration. They were then centrifuged in groups of four and the supernatant fluid pipetted off. The following experiment serves as an example (concentration of the colouring fluid: 0·478 grm. Hb per 100 c.c.):

Table I.

No.	Time of colouring (min.)	Hb in grm. per 100 c.c. of supernatant fluid
1	¼	0·26
2	½	0·25
3	¾	0·18
4	1	0·21
5	1½	0·19
6	2	0·15
7	2½	0·14
8	3	0·14

In the above results there is a fairly constant decrease in the Hb concentration of the supernatant fluid, and therefore an increase in the colouring of the tissue, up to 2 minutes. These data correspond with other experiments, including observations of longer duration (up to 15 minutes).

(*b*) *Influence of concentration of* Hb-*solution.* After having washed and decolorized the same amount (1 grm.) of the tissue in each of a set of centrifuge-tubes, it was again coloured by adding to each portion the same amount of Hb but in varying dilution. After centrifuging the colouring-fluid was poured off and the tissue washed with distilled water until the water used contained no Hb. It was then decolorized with 1 p.c. NaCl and the Hb content of this fluid was determined. Table II shows some of the results.

TABLE II.

No.	Hb in grm. per 100 c.c. of solution	Volume of solution (c.c.)	Hb in mg. per grm. of tissue
1	0·12	40	31·6
2	0·12	40	31·6
3	0·179	30	29·9
4	0·239	20	31·3
5	0·478	10	31·8

Evidently the quantity of Hb absorbed by the tissue from solutions of the strengths employed is independent of the concentration. In another series of experiments even colouring with the undiluted standard solution (23·9 grm. Hb per 100 c.c.) gave the same results as obtained with a colouring-fluid diluted a hundred times. It is true that directly after colouring the tissue seemed to be much more intensively coloured, but the greater part of it could be removed by washing with water.

(*c*) *Influence of temperature.* Centrifuge-tubes, each with 1 grm. of decolorized and washed tissue, were brought up to the required temperature in a water bath. Hb-solution was then added, and after shaking and centrifuging the fluid was again poured off and the tissue then washed with distilled water. Hb-solution and distilled water were brought up beforehand to the required temperature. After centrifuging decolorization with NaCl took place at room temperature.

TABLE III.

No.	Temperature (° C.)	Hb in mg. per grm. of tissue
1	10·0	22·6
2	19·5	22·1
3	27·5	24·2
4	36·0	23·3
5	40·0	19·1

According to the data given above, it does not seem as if temperature has any influence. Other experiments gave the same results within a range from 10° to 40° C.

(*d*) *Influence of salt concentration.* Even in the preliminary experiments the great influence of salts on the colouring process manifested itself, and as the influence of NaCl, KCl and $NaHCO_3$ seemed to be about equally marked, it was decided to use NaCl for the study of the influence of concentration of salt on the colouring of tissue with Hb. Portions of decolorized and washed tissue were coloured again with solutions containing the same amount of hæmoglobin (0·478 grm. per 100 c.c.) but different amounts of salt. After centrifuging and washing with distilled water the tissue was decolorized with 1 p.c. NaCl and the Hb-content of this fluid determined. As an example the data of an experiment are given in Table IV, to which corresponds Fig. 1.

TABLE IV.

No.	NaCl in grm. per 100 c.c. Hb-solution	Hb in mg. per grm. of tissue
1	0·0	47·0
2	0·02	42·4
3	0·1	15·3
4	0·3	9·0
5	0·5	4·4
6	0·8	3·5
7	1·0	2·9
8	1·5	2·1

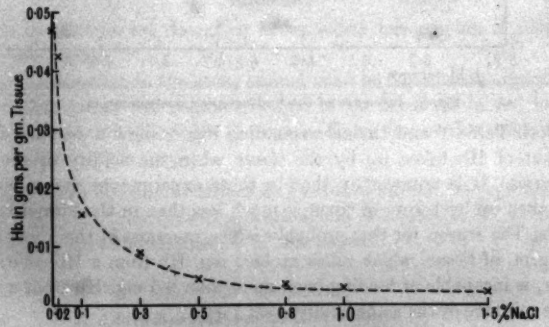

Fig. 1. Influence of salt concentration.

These experiments show the great sensitivity of the colouring process to electrolytes, as even a concentration of 0·02 p.c. NaCl is sufficient to show a marked decrease in colouring.

(e) *Influence of hydrogen-ion concentration.* The colouring fluids in these experiments were prepared from mixtures of phosphates (conc. *N*/60) to which was added the same amount of Hb (0·478 grm. per 100 c.c.).

The pH of the solutions was measured after they had been in contact with the tissue by the antimony method of Brinkman, to whom I am indebted for the determinations. The technique of colouring and decolorization of the tissue was the same as in the former experiments.

TABLE V.

No.	pH of Hb-solution	Hb in mg. per grm. of tissue
1	5·9	12·6
2	6·2	12·5
3	6·4	12·9
4	6·6	10·6
5	6·8	8·5
6	6·9	8·7
7	7·1	9·5
8	7·7	5·2

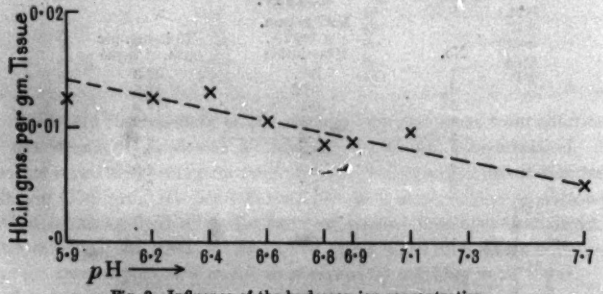

Fig. 2. Influence of the hydrogen-ion concentration.

Both Table V and the corresponding Fig. 2 show a decrease in the amount of Hb taken up by the tissue when the acidity of the fluid decreases. It is noteworthy that in these experiments the amount of Hb taken up by 1 grm. of tissue is much less than in the former experiments. The reason for this probably is the presence of the phosphates, as 1 grm. of tissue, which takes up 38·2 mg. Hb from a Hb-solution in water, is incapable of holding back more than 9·5 mg. Hb from a phosphate mixture of the same acidity (pH 7·1).

DISCUSSION.

The experiments mentioned above show that under certain conditions minced muscle tissue may take up Hb from a solution. The conditions under which this takes place seem to point to the phenomenon being

due to adsorption. This adsorption is greatly influenced by the presence of electrolytes, but to a much less extent by alteration in acidity, although the results obtained with C(H) varying are somewhat affected by the electrolytes present.

The effect of electrolytes on the adsorption makes it improbable that it occurs *in vivo*, and therefore the muscle hæmoglobin must be supposed to be in solution in the tissue fluid. This supposition is borne out by the fact that if all the fluid is pressed out of the tissue in a Buchner press the tissue becomes quite colourless.

Although probably of no importance *in vivo*, this adsorption may well play a part in some of the methods used for the determination of muscle hæmoglobin. The use of distilled water either for perfusion of the blood vessels after bleeding or for extraction of the minced tissue must be regarded as unsuitable, as part of the hæmoglobin may become adsorbed. The best way to remove the hæmoglobin in the vessels is perfusion with Ringer's fluid and rigorous massage of the muscles. After that the muscle tissue may be cut up as finely as possible to remove the pigment from the muscle, for this purpose scissors should not be used, as when they are it is very difficult to get an evenly distributed muscle pulp. Extraction of the muscle pulp may be done with Ringer or with a 1 p.c. NaCl solution and should leave the muscle pulp colourless after 5–10 minutes.

SUMMARY.

The conditions are described under which hæmoglobin is adsorbed by muscle tissue.

Such adsorption in the living animal must be regarded as improbable, but it may affect the results of some of the methods in use for the determination of hæmoglobin in muscle. Extraction with a salt solution is suggested for this purpose.

My thanks are due to Messrs J. Pieterse and G. Osinga for their assistance in the numerous experiments, and also to Mr D. J. Rossouw for his help with the translation of this paper.

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THE EFFECT OF TEMPERATURE ON THE EQUILIBRIUM OF CARBON DIOXIDE AND BLOOD AND THE HEAT OF IONIZATION OF HÆMOGLOBIN.

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(*From the Physiological Laboratory, Cambridge.*)

I. *Introduction.*

The diminution in the amount of carbonic acid combined in the blood caused by an increase in temperature from 15° to 38° C. has been discussed by Stadie and Martin(1), who have given references to earlier work on this problem. In this paper the range of temperatures investigated has been extended by the determination of the amount of carbon dioxide combined at different pressures at 0° C., as well as at 15° and 38°. The observations on blood have been supplemented by measurements on purified hæmoglobin solutions at 0° C. and at body temperature. Measurements on hæmoglobin solutions are not affected by the formation of lactic acid, a secondary reaction which may interfere with the application of thermodynamical formulæ to a system like the blood.

The experiments of Stadie and Martin(1) indicated that the effect of temperature on the carbon dioxide equilibrium could be predicted from the heat of ionization of the protein by van't Hoff's formula for mobile equilibrium, but the more recent experiments of Stadie, Austin and Robinson(2) indicate that the heat of ionization calculated by the formula is from 7000–8000 calories, whereas the heat determined by the calorimetrical methods of Meyerhof(3) is from 11,000–13,000 calories.

Our observations on blood and on hæmoglobin solutions confirm the lower values obtained by Stadie, Austin and Robinson. A theoretical explanation of the difference between observed and calculated heats of reaction will be discussed.

II. *Methods.*

In each determination 3 c.c. of sheep's blood has been equilibrated with a mixture of air and carbon dioxide in a Barcroft tonometer. The total pressure inside the tonometer has been measured with a small mercury manometer, and the partial pressure of carbon dioxide has been

calculated from the gas analyses made with Haldane's apparatus. The carbon dioxide in the blood has been determined by van Slyke's constant volume apparatus.

III. *The carbon dioxide dissociation curves of oxygenated blood.*

Measurements of the amounts of carbonic acid in blood at different tensions of carbon dioxide have been recorded in Fig. 1. The three curves marked 0° C., 15° C. and 38° C. show that an increase in temperature caused a very considerable diminution in the amount of carbon dioxide in blood, and, in this respect, carbon dioxide resembles oxygen (see Barcroft and King(4), Brown and Hill(5), Adair(6)).

Nevertheless, there is a difference between the two gases, because the effects of variations in temperature on the oxygen dissociation curve can be represented by an alteration in a single constant, whereas the alteration in the carbon dioxide dissociation curve cannot be represented in this simple manner.

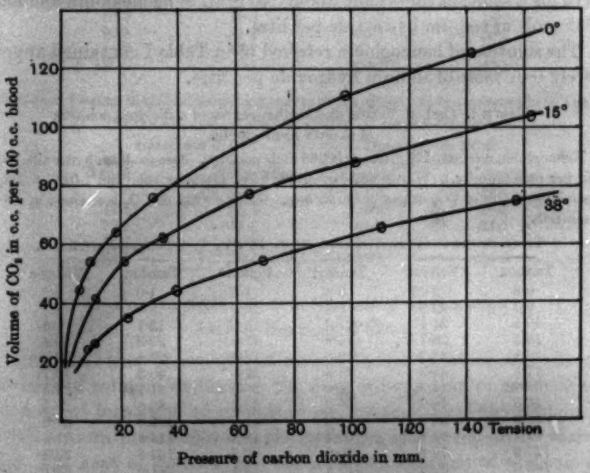

Fig. 1. Carbon dioxide dissociation curves of oxygenated sheep's blood at 0° C., 15° C. and 38° C. Volumes in c.c. of CO_2 (dissolved and combined) per 100 c.c. of blood. Pressures in mm. of mercury partial pressure of CO_2 in the gas phase.

Our measurements cover a wide range of pressures of carbon dioxide, including the region of pressures higher than the physiological investi-

gated by Straub and Meier(7, 8), and Parsons and Parsons(9). Straub and Meier reported the existence of discontinuous changes in the slope of the curve, and Parsons and Parsons have suggested that the discontinuous changes are due to the precipitation of hæmoglobin. Our results differ from the observations of Straub and Meier, in that points lie on smooth curves, and we find no evidence for sudden changes in the capacity of hæmoglobin to combine with acids or bases. The technique of Straub and Meier has been criticized by Warburg(10).

IV. *The carbon dioxide dissociation curves of hæmoglobin solutions.*

The observations recorded in Tables I, II and III show that effects of temperature changes on the carbon dioxide dissociation curves of blood are paralleled by similar changes in a simpler system, namely a mixture of hæmoglobin and sodium bicarbonate.

In a previous paper (Adair(11)) it has been pointed out that the dissociation curve for blood at 38° is almost exactly the same as the curve for a solution containing about 140 grm. of hæmoglobin and about 0·035 mols of sodium hydroxide per litre.

The solution of hæmoglobin referred to in Table I contained approximately 0·01 mols of sodium hydroxide per litre.

TABLE I. Carbon dioxide dissociation curves of a dialysed solution of sheep's hæmoglobin.

Hæmoglobin content, 176 grm. = 0·00263 mols per litre. Base (sodium hydroxide) = 0·01 mols per litre (approx.). Water, 867·7 grm. per litre. (Density solution, 1·0437.) Tension = partial pressure of CO_2 in gas phase in mm. Volume = c.c. of CO_2 contained in 100 c.c. of solution.

Temperature 0° C.		Temperature 15° C.		Temperature 38° C.	
Tension	Volume	Tension	Volume	Tension	Volume
0·8	2·05	0·3	1·5	1·0	1·2
8·7	20·8	8·6	10·5	8·0	5·5
10·1	21·1	11·0	13·6	12·7	6·6
16·5	28·7	15·8	17·0	20·3	9·4
26·9	35·2	19·6	19·6	29·8	12·2
27·3	35·2	25·4	22·6	40·2	15·7
34·6	41·3	34·4	26·9	64·1	21·5
46·5	48·7	50·2	33·2	83·6	25·7
63·9	56·0	75·9	41·7	102·0	28·9
92·1	62·9	98·4	48·8	114·8	31·4
127·2	76·8	150·0	60·2	155·5	39·9
145·0	80·8	217·8	73·4	241·0	52·1
179·3	89·5				
221·3	101·3				

The measurements on concentrated solutions of hæmoglobin have been supplemented by a series of experiments on dilute solutions. The degree of accuracy of the observations with dilute solutions is less than

that obtained with the concentrated solutions. As a first approximation, it appears that the effect of temperature on the curves for the dilute solution is the same as its effect on more concentrated solutions.

The following tables illustrate the effect of temperature on the dissociation curve of a solution of oxyhæmoglobin containing 5·35 grm. of protein per litre. The content of sodium hydroxide is the same in each experiment in Table II, but the absolute value of the concentration of sodium hydroxide has not been determined.

TABLE II. Carbon dioxide dissociation curves of dilute solutions of sheep's hæmoglobin.

Hæmoglobin content, 53·5 grm. = 0·0008 mols per litre. Tension = pressure of CO_2 in the gas phase. Volume = c.c. of CO_2 contained in 100 c.c. solution.

Temperature 0° C.		Temperature 38° C.	
Tension	Volume	Tension	Volume
5·4	8·5	8·2	3·4
12·3	11·8	10·5	4·6
20·0	15·3	23·3	6·9
42·9	22·3	42·5	10·8
69·3	30·5	79·0	14·7
95·6	37·5	109·8	20·8
148·2	47·7	151·5	23·5
180·5	56·7	200·2	27·1

TABLE III. Carbon dioxide dissociation curves of the dilute solution referred to in Table II, with 0·065 grm. of $NaHCO_3$ added to 100 c.c. solution.

Temperature 0° C.		Temperature 38° C.	
Tension	Volume	Tension	Volume
5·0	28·3	7·6	20·8
18·4	36·6	20·8	26·0
49·4	48·1	51·8	32·1
96·7	61·3	110·0	40·8
134·7	70·2	152·6	45·2

V. *The reactions affecting the heat of combination of carbon dioxide and blood.*

The total heat of reaction of carbon dioxide in blood depends upon the heat of solution of the gas, the heat of ionization of carbonic acid, the heat of formation of unionized protein-acid, from the protein ions of the sodium proteinates and the hydrogen ions given off by carbonic acid. The heat of solution and the heat of ionization of carbonic acid are known, and if there are no subsidiary reactions it is possible to determine by experiment the heat of ionization of the protein.

It is necessary to consider briefly the evidence for the existence of subsidiary reactions which may be a source of error in calculations of the heat of ionization of the protein. In the first place, lipoids in blood

increase the solubility of carbon dioxide by about 4 p.c., as stated by van Slyke and others(12). In the second place, hæmoglobin depresses the activity coefficient of bicarbonate. Unfortunately we have no evidence concerning the effects of temperature on this reaction, but in the case of chlorides and phosphates at 0° C. the fall in the activity coefficients determined by one of us appears to be comparable with the effects at body temperature observed by van Slyke and others(13), as stated in a previous paper (Adair(14)).

Buckmaster(15) and Henriques(16) have formulated the hypothesis that the carbonic acid in blood combines with hæmoglobin, but, in view of the fact that the red corpuscle differs in certain respects from an infinitely dilute solution, extensive mathematical and experimental investigations are required before we can hope to distinguish between the effects due to the interionic forces described by Debye and Hückel(17) and the effects due to true chemical combination, if any combination takes place. Stadie and Hawes(18) have made an extensive investigation in which they have not found it necessary to assume the existence of a chemical compound of hæmoglobin and carbon dioxide. In the absence of any certain information concerning the composition and properties of the possible compound, our calculations have been based on the assumption that all of the combined carbonic acid in blood is in the form of bicarbonate, in accordance with the work of Stadie and his colleagues.

VI. *The hydrogen-ion concentrations in blood and hæmoglobin solutions at different temperatures and the heat of ionization of hæmoglobin.*

The hydrogen-ion concentration or the pH of blood and hæmoglobin solutions has been calculated by Hasselbalch's formula,

$$p\mathrm{H} = pK + \log [\mathrm{HCO_3}]/[\mathrm{H_2CO_3}] \qquad \ldots\ldots(1).$$

$[\mathrm{H_2CO_3}]$ = the concentration of free carbonic acid.

$[\mathrm{HCO_3}]$ = the concentration of bicarbonate ions.

pK = $-\log K$, where K is the apparent dissociation constant of carbonic acid.

The concentration $[\mathrm{H_2CO_3}]$ in millimols per litre at the temperature T has been calculated from the known value of α, the solubility coefficient of carbon dioxide in water at temperature T, by the formula

$$[\mathrm{H_2CO_3}] = p\left(\alpha.w.\frac{1}{760}.\frac{100}{2{\cdot}24}\right).$$

p = pressure of carbon dioxide in mm. of mercury and w = c.c. of water per c.c. of solution.

The constant pK at 38° C. has been estimated from the measurements of Stadie and Hawes(17). The values of pK at 15° and 0° have been calculated by van 't Hoff's formulæ for mobile equilibrium referred to below, on the assumption that the heat of ionization of carbonic acid is 2800 calories. The concentration of bicarbonate [HCO_3] has been calculated on the assumption that the bicarbonate is equal to the total concentration of CO_2 minus the dissolved carbonic acid.

Reference must be made to Warburg's(10) paper for a detailed thermodynamical discussion of Hasselbalch's formula. In considering differences in pH rather than the absolute values, the corrections introduced by Warburg tend to cancel out, and for the purpose of the approximate calculations given in this work it seems justifiable to shorten and simplify the theoretical formulæ by the use of the law of mass action in its original form. The semi-empirical constants used in applying the formula are given in Table IV.

TABLE IV.

Temperature (0° C.)	pK blood	pK Hb	a water	$0{\cdot}0588a.w$ blood	$0{\cdot}0588a.w$ Hb sol.
0	6·42	6·43	1·713	0·0926	0·0873
15	6·31	6·32	1·019	0·0551	0·0518
38	6·15	6·16	0·555	0·030	0·0283

Warburg has shown that if the bicarbonate concentration is plotted as ordinate and the pH as abscissa, the most reliable observations on blood and serum lie on a straight line. In our experiments the points lay on straight lines over a considerable range of hydrogen-ion concentrations, and from these lines we have read of the values of the pH corresponding to a constant concentration of bicarbonate.

TABLE V. pH at different temperatures at a constant concentration of bicarbonate.

Solution	[HCO_3] millimols	pH 38°	pH 15°	pH 0°	$\frac{\Delta pH^*}{\Delta T}$
Blood	20·0	7·31	7·66	7·93	−0·00163
Blood	30·0	6·90	7·24	7·48	−0·00153
Hb 17·5 p.c.	7·5	6·80	7·20	7·50	−0·00184
Hb 17·5 p.c.	10·5	6·70	7·06	7·35	−0·00171
Hb 5·35 p.c.	15·6	6·70	—	7·22	−0·00137
Hb 5·35 p.c.	15·5	7·37	—	8·00	−0·00166

* $\Delta p H/\Delta T$ is the average value of the variation in pH per degree from 0° C. to 38° C.

Under the conditions where the concentration of sodium bicarbonate is constant, the total base is also constant and the total hæmoglobin is

constant, and therefore the degree of ionization of the protein is theoretically constant. Stadie and Martin(1) have pointed out that if the degree of ionization of an acid at two different temperatures is a constant, the heat of ionization can be calculated from the concentrations of hydrogen ions by van 't Hoff's isochore, restated below:

$$\frac{\partial \log [H]}{\partial T} = \frac{-\partial pH}{\partial T} = \frac{Q}{2 \cdot 303 RT^2} \qquad \ldots\ldots(2).$$

Q = the heat absorbed by the ionization of 1 grm. mol of hydrogen ions at the absolute temperature T.

The integration of this formula on the assumption that Q is a constant leads to the equation given below:

$$Q = 24{,}017 \, (pH_0 - pH_{15}) = 17{,}842 \, (pH_{15} - pH_{38}) \quad \ldots\ldots(3).$$

pH_0, pH_{15} and pH_{38} denote the values of the pH at 0° C., 15° C. and 38° C. recorded in Table V.

Calculations of Q made by these formulæ have been made and recorded in Table VI.

TABLE VI. Heat absorbed by the ionization of one equivalent of hæmoglobin.

Solution	$[HCO_3]$	pH	$\frac{\partial pH}{\partial T}$	$\frac{\partial pH}{\partial T}$	Q	Q
	Millimols per litre	At 38°	Range 0°–15°	Range 15°–38°	Range 0°–15°	Range 15°–38°
Blood	30	6·90	−0·016	−0·015	5800	6100
Blood	20	7·31	−0·017	−0·016	6200	6500
Hb 17·5 p.c.	10·5	6·70	−0·019	−0·016	7000	6400
Hb 17·5 p.c.	17	6·80	−0·020	−0·017	7200	7200

VII. *Comparison of the theoretical values of the heat of ionization and the values obtained by calorimetrical methods.*

The heat of ionization of albumin is 12,400 calories, according to the calorimetrical determinations of Meyerhof. The heat of ionization of blood proteins is 10,500 calories, according to the calorimetrical measurements of Brown and Hill(5).

The theoretical values of the heat of ionization calculated by formula (2) can be divided into two groups. The observations of Stadie and Martin on human blood, and Stadie, Austin and Robinson on a specimen of separated serum, lead to values of 10,000 calories and 8900 calories, whereas the observations of Stadie, Austin and Robinson(2) on dog's blood lead to lower values, namely 7200, 7760 and 7550. Austin, Sunderman and Camack(19) have made observations on alligator serum which are consistent with the figure of 7400 for the heat of ionization. Including the measurements recorded in Table VI, the

majority of the experiments indicate that the theoretical value of the heat of ionization is less than that obtained by calorimetrical methods. The simplifications in the theoretical formulæ may be responsible for a part of the discrepancy, but it seems probable that the difference between the observed and the calculated heats of reaction is mainly due to the fact that an amphoteric electrolyte can increase the hydrogen-ion concentration by two distinct mechanisms. Firstly, it can ionize as an acid, and this heat of ionization may be represented by the symbol Q_a. Secondly, the hydrogen-ion concentration can be increased if water is ionized and the hydroxyl ions combine with the amphoteric electrolyte. The heat absorbed in this process is equal to $Q_w - Q_b$, where Q_w is the heat of ionization of water and Q_b is the heat absorbed by the ionization of the electrolyte as a base.

If it is assumed that x mols of hydrogen ions are due to the ionization as an acid and $(1 - x)$ mols are due to the ionization of water, the total heat per mol, Q, is then given by formula (4):

$$Q = xQ_a + (1 - x)(Q_w - Q_b) \qquad \text{.....(4).}$$

According to Meyerhof's experiments on proteins, Q_a is about 12,350 calories, Q_b is about 13,000 calories and Q_w is about 13,720 calories.

The term $(Q_w - Q_b)$ is relatively small and therefore the low values for the total heat Q calculated from the measurements of Stadie, Austin and Robinson and the observations recorded in Table VI can be accounted for on the assumption that a part of the hydrogen ions are due to the ionization of the protein as a base and the ionization of water.

Theoretically the values of Q calculated by formula (2) should be of service in estimations of the fractions x and $(1 - x)$ referred to above, but in practice there are other factors which must be taken into account, and the estimation of these fractions must be deferred until more data are available.

There is however one special case which deserves consideration, namely the isoelectric point, where the ionization of the protein as an acid is equal to its ionization as a base. Under these conditions $x = 0{\cdot}5$, and $Q = 0{\cdot}5\,(Q_a + Q_w - Q_b)$. Meyerhof's determinations referred to above indicate that at the isoelectric point Q is about 6500 calories, a figure of the same order as some of the observations recorded in Table VI. If Q is equal to 6500, the pH at the isoelectric point diminishes by 0·018 units per degree over the range from 0° to 15° C. and it diminishes by 0·016 units per degree over the range from 15° C. to 38° C.

Although it is not yet possible to make a quantitative application

of the formula $Q = xQ_a + (1 - x)(Q_w - Q_b)$, there is one qualitative conclusion which must be considered.

A diminution in the concentration of hydrogen ions (or an increase in the pH) should increase the value of x, and it follows that the terms Q and $\partial p\mathrm{H}/\partial T$ must be increased by an increase in pH. If Q is a function of the hydrogen-ion concentration, the term β in Van Slyke's buffer equation restated below must be a function of the temperature rather than a constant, as suggested by Stadie and Martin.

$$B = \beta(p\mathrm{H} - I) \qquad \text{.....(5).}$$

B = number of atoms of base combined with one equivalent of hæmoglobin, β = a constant, designated the "buffer value," I = the pH at the isoelectric point.

If I is a function of the temperature and β is a constant independent of the temperature, it is easy to show that Q must be independent of the pH. Stadie, Austin and Robinson state that in two experiments β increased when the temperature increased from 20° to 38°, in one experiment β remained constant and in one experiment it diminished. Our experiments indicate that β is increased by an increase in temperature.

VIII. *The theoretical significance of the heat of ionization of amino-acids and proteins.*

In previous work on the heat of ionization of amino-acids and proteins, the results have been stated in terms of Walker's theory of amphoteric electrolytes.

The heat Q_1 given by the reaction in formula (6) has been regarded as a "neutralization heat," and the heat Q_a given in formula (7) has been regarded as the heat of ionization of the protein as an acid.

$$NH_2RCOOH + NaOH = NH_2RCOO^- + Na^+ + H_2O + Q_1 \text{.....(6).}$$

$$NH_2RCOO^- + HCl + Na^+ = NH_2RCOOH + NaCl + Q_a \text{.....(7).}$$

The sum $Q_1 + Q_a = Q_w$ the heat of ionization of water. Meyerhof found that in the case of glycocoll, $Q_1 = 2800$ calories and $Q_a = +11{,}450$ calories. Q_a is very much larger than the heat of ionization of the carboxyl group in acetic acid (about 600 calories), and Meyerhof explained this difference by the hypothesis that amino-acids form complex salts.

Harris(20) has shown that the titration curves of amino-acids and

proteins are consistent with Bjerrum's theory[1] that the neutral amino-acid exists as a doublet ion represented by the formula $^+NH_3RCOO^-$. It is necessary to reconsider the significance of the measurements of the heats of reaction from the point of view of this theory.

The reaction between the doublet ion and sodium hydroxide can be represented by the formulæ (8) and (9) below:

$$^+NH_3RCOO^- + NaOH = (OH)NH_3RCOO^- + Na^+ + Q_b \quad \ldots\ldots(8).$$

$$OH.NH_3RCOO^- = NH_2RCOO^- + H_2O \quad \ldots\ldots(9).$$

If it is assumed that the formation of water by the reaction in formula (9) takes place without any evolution or absorbtion of heat, Q_b in formula (8), or the heat absorbed by the ionization of the amino-acid as a base is the same as Q_1 in formula (6). The heat of ionization of ammonium hydroxide is about 1500 calories, and it is interesting to observe that this figure is of the same order of magnitude as the values of Q_1 observed by Meyerhof, who obtained 2900 calories for glycocoll and 1370 calories for albumin.

If the reaction between the sodium salt of the amino-acid and hydrogen chloride is represented by formula (10):

$$OH.NH_3.RCOO^- + Na^+ + HCl = {}^+NH_3R.COO^- + NaCl + H_2O + Q_2 \quad \ldots\ldots(10).$$

$Q_2 = Q_w - Q_b$, or 13,700 calories minus about 1500 calories. The difference, 12,200 calories, is approximately equal to the experimental determinations of the heat evolved on the addition of hydrochloric acid to sodium proteinates.

Although both points of view are consistent with the experimental evidence, the hypothesis that the neutral protein exists as a doublet ion enables us to predict that the heat of reaction of a sodium proteinate with hydrochloric acid should be about 12,000 calories, on the assumption that the heats of ionization of the amino groups and carboxyl groups are about the same as the heats of ionization of ammonium hydroxide and acetic acid, whereas the theory adopted by Brown and Hill(5), Stadie and Martin(1) and Meyerhof(3) appears to require the assistance of supplementary hypotheses in order to explain this heat of reaction.

[1] After this paper had been sent to the press our attention was drawn to applications of Bjerrum's theory to the heats of ionization of other ampholytes, made by previous workers. Glycine, for example, has been studied by Kolthoff and Tekelenburg, *Rec. Trav. Chim. Pays-Bas*, 46. p. 33. 1927.

SUMMARY.

The carbon dioxide dissociation curves of blood and of hæmoglobin solutions have been measured at three different temperatures, 0° C., 15° C. and 38° C.

The heat of ionization of hæmoglobin calculated from these observations is less than that observed by calorimetric methods. The discrepancy can be explained on the assumption that the heat is partly due to the ionization of carboxyl groups and partly due to the ionization of water and the combination of hydroxyl ions with the protein. The fraction of the heat due to the ionization of carboxyl groups is a function of the *p*H, and in the calorimetrical measurements recorded the *p*H is greater than that which obtains in the blood.

The magnitude of the heat of ionization indicates that neutral proteins may exist as doublet ions.

We wish to thank Prof. Barcroft for his assistance in this work.

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THE CORONARY CIRCULATION.

II. The effect of changes of temperature and of heart rate.

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The experiments described here were performed with a method, the details of which have been given in earlier communications (1, 2). The method consists in establishing a perfusion of a large branch of a coronary artery at constant pressure, and in measuring the inflow of blood into the perfused artery by a hot-wire anemometer and a continuous method of registration. The separation of the coronary circulation in the perfused area from the systemic circulation enabled us to study the changes in the coronary blood flow which follow variations of the aortic pressure and of the output of the heart, in so far as these variations affect the strength of the cardiac contractions (3). It is obvious that, since in these experiments the coronary system is supplied with blood from two independent sources, we can investigate the effects of various agencies either by their direct administration to the perfused area, or by their administration to the rest of the cardiac muscle through the coronary supply of the aortic origin. In view of the contradictions which exist as regards the effect of temperature on the coronary circulation, we carried out a series of experiments with the object of separating the effects due to the direct influence of temperature on the coronary blood vessels from those which may be produced indirectly as the result of changes in the strength of contraction and in the rate of the heart beat.

The direct effect of temperature on the coronary blood vessels.

Cruickshank and Subba Rau (4) found that isolated coronary arteries relax on cooling and contract vigorously on warming, the maximal contraction taking place at a temperature of 37°–40° C. Our first experiments were performed in order to find out whether the observation of these authors holds true for arteries when they are *in situ*. The experiments were performed as follows. The temperature of the

systemic blood of a heart lung preparation was kept constant throughout the period of observation; the temperature of the blood perfusing the coronary artery was made to vary between 40° C. and 29° C. The systemic output of the heart was usually kept at 500–700 c.c. per minute, so that the administration of 15–30 c.c. of cooled or warmed blood from the perfused area did not materially influence the temperature inside the heart; and in fact the temperature of the general mass of blood could scarcely have altered since on changing the temperature of the perfusing blood by about ten degrees, the spontaneous heart rate changed by not more than a few beats per minute. The temperature of the blood perfusing the coronary artery was measured in the cannula inserted into the artery. Therefore the readings of the thermometer indicated the temperature of the blood in the first part of the perfused artery only; on penetrating the heart muscle the blood gradually assumed the temperature of the heart, which in our experiments was in contact with a large amount of warm systemic blood, and was also warmed by radiant heat from the outside. We endeavoured to obtain information as to the extent of changes in the temperature of the perfused part of the heart by means of thermo-electric junctions, which were inserted so as to penetrate about half way through the wall of the perfused part of the ventricle. It was found that, on lowering the temperature of the perfusing blood from 39° C. to 29° C., the systemic blood being kept at 36° C., the temperature of the muscle dropped in 5 min. from 37·5° C. to 36·9° C., and in 10 min. to 35·8° C. On the other hand, when the temperature of the systemic blood was lowered from 40° C. to 30° C., the perfusing blood being kept at 36° C., the temperature of the muscle dropped from 39° C. to 31·2° C. in about 5 min. Thus, the lowering of the temperature of the perfusing blood causes cooling mainly of the first part of the perfused artery, while the lowering of the temperature of the systemic blood and of the outside of the heart causes cooling mainly of the cardiac muscle and the smaller blood vessels. Table I shows the effect of cooling the blood perfusing the left coronary artery on the blood flow through the artery.

The changes in the coronary blood flow on cooling or warming the blood are greater when the changes of temperature are rapid; where they are slow, the effect tends to be obscured, as will be explained later.

Analysis of the direct effect of temperature on the coronary blood vessels by the hot-wire method shows that the increase in the blood flow at low temperatures is due to dilation of the coronary blood vessels. Fig. 1 gives two superimposed hot-wire records which were taken at a

TABLE I.

Heart lung preparation. The temperature of the systemic blood, the mean aortic pressure, the coronary perfusion pressure and the output of the heart were kept constant; the heart rate was controlled at 140 beats per minute.

Temperature of the perfusing blood in ° C.	Blood flow through the perfused left coronary artery in c.c. per min.	Remarks
38·5	20·3	Average for 5 min.; variations from 20·0–20·7 c.c.
38·0–34·5	20·7–23·1	Fall of temperature during about 4 min.
34·0–30·0	25·0–27·3	
30·0	27·3	Eight successive measurements
30·0–33·0	27·3–25·0	Rise of temperature during about 4 min.
36·0–39·5	23·1–18·5	
39·5	18·6	Average of 4 measurements
39·0–30·0	18·6–28·2	Rapid fall of temperature
30·0–29·0	29·2–31·6	
29·0–36·0	31·6–25·2	Rapid rise of temperature
36·0–38·0	25·0–22·7	

Fig. 1. Superimposed hot-wire records of the inflow of blood into the perfused left coronary artery: (*a*) base line of the coronary blood flow, (*b*) optical record of the aortic blood-pressure, (*c*) original hot-wire record taken during the perfusion of the artery with blood at 38·5° C., (*d*) retraced hot-wire record during the perfusion with blood at 31° C. The aortic blood-pressure, the systemic output and the heart rate were maintained constant. This and the rest of the tracings are to be read from left to right. Time in all records—0·04 sec.

low and a high temperature of the perfusing blood. It can be seen that, while the systolic arrest of the coronary inflow was complete at low and at high temperatures, the inflow during diastole was greatly increased at low temperatures.

These experiments show that the coronary blood vessels react to changes in temperature in the beating heart in the same manner as isolated coronary rings, and that the increase in the inflow of blood is

due to a loss of tone by the perfused artery and not to changes in the contractions of the heart muscle.

The indirect effect of temperature on the coronary blood vessels.

The effect of cooling or warming the heart muscle on the circulation through the perfused area is the reverse of that of cooling or warming the perfusing blood. The lower the temperature of the heart, the smaller is the coronary blood flow. Table II illustrates the magnitude of this effect.

TABLE II.

The heart rate was controlled at 140 beats per minute. The mean aortic pressure, the coronary perfusion pressure, the temperature of the perfusing blood and the output of the heart were kept constant.

Temperature of the systemic blood in ° C.	Blood flow through the perfused left coronary artery in c.c. per min.	Remarks
37	18·2	
32	16·9	Fall of temperature in about 5 min.
30	15·4	
36	16·0	
38	17·4	Rise of temperature in about 6 min.
39	20·7	
30	15·9	Rapid fall of temperature

The effect of changing the temperature of the systemic blood is less obvious when the heart rate is not controlled. The reason for this becomes clear if Fig. 2 is considered in detail. The driving of the heart was started at the point marked *A*, when the temperature of the systemic blood was 39° C. In consequence of the artificial stimulation the heart accelerated by a few beats, while the coronary inflow remained unaltered. The temperature of the systemic blood was then lowered, and the driving was discontinued from *B* to *C*. This caused an immediate slowing of the heart, and a considerable augmentation of the blood flow through the perfused coronary artery. It is therefore clear that, if the heart rate is allowed to change, the effect of temperature will be diminished. In the experiment from which Fig. 2 was constructed a drop of temperature from 39° C. to 30° C. diminished the blood flow through the perfused artery in the driven heart from 24 c.c. to 17 c.c. per minute, whereas in the spontaneously beating heart the same change of temperature diminished the flow only from 24 c.c. to 21 c.c. per minute.

From the consideration of the opposite effect of changes in the temperature of the perfusing blood and of the heart muscle it becomes clear that in experiments in which cooling of the perfusing blood is

prolonged, so that the temperature of the perfused part of the muscle becomes considerably reduced, the direct effect of low temperature on

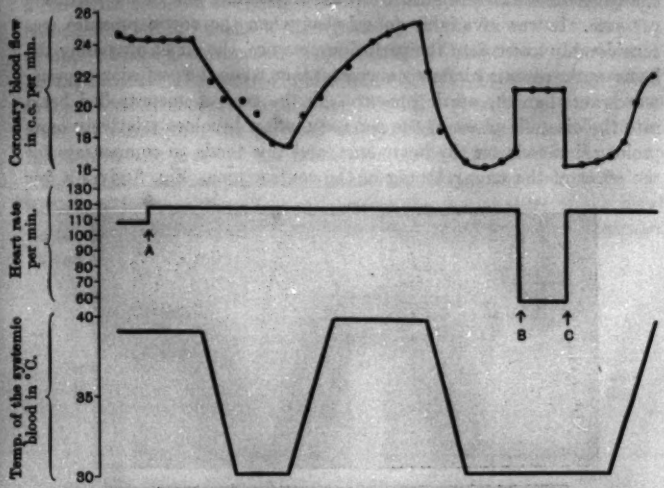

Fig. 2. Effect of varying the temperature of the systemic blood on the inflow of blood into the perfused left coronary artery. The temperature of the perfusing blood was kept constant at 36° C. From *A* onwards the ventricular beat was controlled at the rate of 120 per minute. The driving of the ventricle was interrupted between *B* and *C*.

the coronary arteries is masked by the contrary effect of the low temperature on the heart muscle itself.

The analysis of the effect of changes in temperature of the heart muscles shows that, unlike the case of the direct effect on the coronary arteries, it is the systolic phase of the coronary circulation which becomes altered, while the diastolic phase remains practically unaffected. At a low temperature the contractions of the heart become stronger (5, 6), in consequence of which the diminution of the coronary blood flow during systole becomes more conspicuous. When the contractions are initially strong, cooling of the heart only slightly diminishes the already restricted blood flow through the perfused artery. When the contractions are not strong enough to stop the flow during systole, cooling of the heart has a considerably greater effect because, on account of the strengthening of the contractions, the systolic diminution of the flow becomes more

pronounced. The method used in our experiments allowed us to alter the effect of systole on the coronary inflow within a wide range, by changing the relative heights of the aortic pressure and of the perfusion pressure. It was invariably found that when the aortic pressure was considerably lower than the perfusion pressure, the effect of cooling the heart on the coronary inflow was greater than when the perfusion pressure was lower than the aortic pressure. In the case of uncontrolled heart rate the diastolic phase of the coronary inflow becomes relatively more prolonged the slower the heart rate, and this tends to compensate for the effect of the strengthening of the contractions. Fig. 3 gives a few

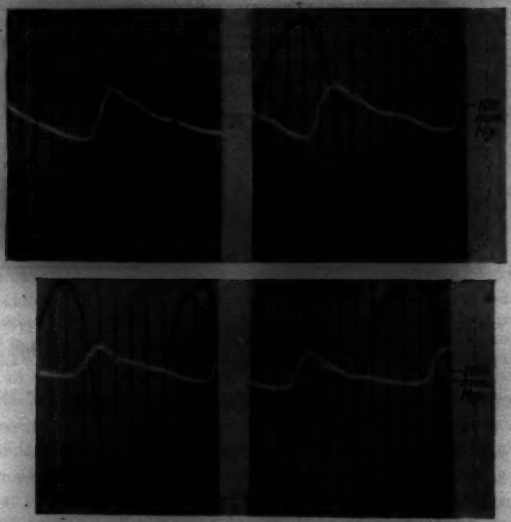

Fig. 3. Original hot-wire records taken at various temperatures of the systemic blood; the blood perfusing the left coronary artery was maintained constant. The ventricular beat was controlled. The temperature was 31° C. in *A* and *C*, 38° C. in *B* and 40° C. in *D*.

hot-wire records of the coronary blood flow taken at different temperatures of the systemic blood in a heart, the rate of which was controlled.

The direct effect of temperature on the coronary blood vessels is more pronounced than the indirect effect just described. In a heart-lung

preparation in which the whole coronary blood is supplied from the aorta, and in which the outflow from the coronary sinus is measured by a Morawitz cannula, lowering of temperature leads to a dilation of the coronary arteries, and to a strengthening of the heart beat; but the complete effect is an increase of the outflow of blood from the sinus (7). In experiments with perfusion of a large branch of the coronary artery it is not strictly accurate to compare the outflow from the coronary sinus with the inflow into the perfused artery, because the sinus collects the blood from the areas of the heart which are perfused as well as from those which are supplied by the aorta. In some experiments the difference between the effect of lowering the temperature of the heart on the perfused artery and on the rest of the coronary system is marked, as can be seen from Table III.

TABLE III.

The temperature of the perfusing blood was kept constant at 36·5° C.
The heart rate was controlled at 130 beats per minute.

Temperature of the systemic blood in ° C.	Inflow of blood into the circumflex branch in c.c. per min.	Outflow of blood from the coronary sinus in c.c. per min.	Mean aortic blood-pressure in mm. Hg.
38	22·5	40·0	
32	18·1	44·5	90
37	21·9	39·3	
39	24·1	53·9	
30	18·1	67·4	100
37	20·4	54·0	

At low temperatures the perfused coronary artery is more strongly compressed by the contractions of the heart, and therefore the blood flow through the artery is diminished; the coronary sinus however also collects the blood flowing from the blood vessels, which are themselves subjected to cooling and are therefore dilated. The diminution of the blood flow through the perfused artery is thus counterbalanced by an augmentation of the circulation in the rest of the coronary system. It has been shown previously that the relation between the sinus outflow and the total coronary blood flow is unaffected by changes of temperature (8). Therefore the results shown in Table III cannot be explained by an alteration in the distribution of the coronary blood between the sinus and the Thebesian blood vessels.

The effect of changes of heart rate on the coronary blood flow.

The consideration of the effect of changes of the heart rate will be started with a comparison of the coronary inflow in the beating ventricle with that in the quiescent or fibrillating ventricle. Langendorff (9),

H. Fredericq (10), and others (11, 12) found that the coronary circulation in the perfused heart is usually increased during ventricular fibrillation. The explanation of this was based on the assumption that the continuous discoordinated contractions of the fibrillating ventricle have a greater massaging action than the coordinated contractions of the beating heart (12). We have confirmed the observations of the previous authors in our experiments, but we do not agree with their interpretation. Table IV shows the effect of ventricular fibrillation on the inflow of blood into the left coronary artery. In each case the fibrillation was induced by a brief stimulation of the ventricle with a strong faradic current which was discontinued as soon as fibrillation started.

TABLE IV.

Effect of ventricular fibrillation on the inflow of blood into the left coronary artery. Each observation was obtained from a different experiment.

Mean aortic blood-pressure before fibrillation in mm. of mercury	Coronary inflow in c.c. per min.		Increase of blood flow in p.c.
	Before fibrillation	During fibrillation	
35	18·7	22·2	19
60	11·1	15·6	41
95	22·3	35·3	58
122	16·7	30·0	79

The higher the mean aortic blood-pressure before ventricular fibrillation the greater is the relative increase of the blood flow through the perfused artery. Both the reason for this, and the mechanism by which the increase of the blood flow is produced, are evident on inspection of Fig. 4, in which the inflow into the left coronary artery was recorded

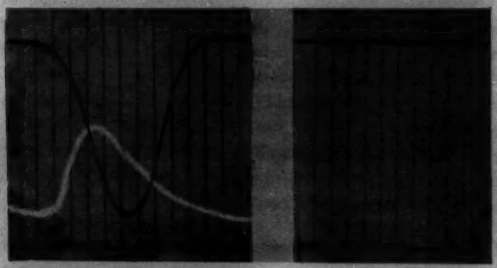

Fig. 4. Effect of ventricular fibrillation on the inflow of blood into the perfused left coronary artery. *A*, the last contraction before ventricular fibrillation. *B*, 5 sec. after the onset of fibrillation.

by a hot-wire anemometer. Record *A* is that of the last contraction of the ventricle, and record *B* was taken 5 sec. later, during ventricular fibrillation. The fibrillating ventricle is unable to compress the coronary blood vessels and therefore the inflow of blood proceeds at the same rate as during the periods of diastole before the fibrillation. In a beating heart the effect of systole on the coronary blood flow is more conspicuous when the cardiac contractions are strong(3); on lowering the aortic blood-pressure the contractions of the heart become weaker, and the coronary circulation becomes less restricted by the systole, and therefore the augmentation of the blood flow during ventricular fibrillation is not as great in this case as during fibrillation induced in a vigorously contracting ventricle. When the cavities of the fibrillating ventricle are kept empty the inflow of blood into the perfused coronary artery remains constant. If however the heart is allowed to fill so that the intraventricular pressure rises and causes compression of the coronary blood vessels, the inflow rapidly diminishes. But as soon as the heart is emptied the inflow of blood returns to the original level. It must be concluded that ventricular fibrillation favours the coronary circulation only by removing the restricting effect of systole; it does not actively assist in the propulsion of blood through the coronary system.

It has been mentioned in a previous communication that, under normal conditions of tone of the coronary blood vessels, the rate of their refilling during diastole bears no relation to the rate of relaxation of the heart, and the maximum inflow occurs late in diastole. Under the influence of vasodilator substances, or in the case of loss of tone by the coronary blood vessels towards the end of an experiment, the refilling of the coronary system after each systole is accelerated. The vessels now afford the least resistance immediately after they have been emptied by the contracting heart, and therefore the inflow becomes faster during the first part of diastole. The effect may be so great that the maximum rate of inflow takes place during the early part of diastole. In all such cases the augmentation of the coronary inflow caused by ventricular fibrillation is, of course, smaller than under the conditions of normal tone of the blood vessels.

An arrest of the heart in diastole has the same effect upon the inflow into the perfused artery as ventricular fibrillation. In two experiments the heart stopped spontaneously, and in others it stopped after prolonged clamping of both coronary arteries. When the temperature of the heart is kept low, clamping of the arteries occasionally fails to cause fibrillation but leads to cessation of all contractions. After release of the arteries

the standstill may sometimes last as long as 10 min. But the ventricles continue to respond by contraction to single induction shocks, so that periods of prolonged diastolic standstill can be alternated with periods of rhythmic contractions. The records thus obtained are like those obtained during ventricular fibrillation. During the periods of arrest the rate of the coronary inflow is constant and, if there is no overfilling of the heart, the rate is the same as during the periods of diastole of the beating heart.

A similar method was used in experiments upon heart rate. The rate of the spontaneous beat of the ventricle was reduced by cooling the S.A. node, or by cooling the whole heart, or by producing complete heart block. After having reduced the rate of the ventricular contractions by one of these methods, the ventricle was stimulated by single induction shocks at rates ranging from just above the spontaneous rate to rates at which the heart ceased to follow the stimuli with regular contractions. The general result of these experiments is that it is impossible to predict with certainty the effect which acceleration of the ventricular contractions will have on the coronary blood flow. The result may be an augmentation, or a diminution, or there may be no change at all. Analysis by the hot-wire method makes it clear that in each case the effect depends on two factors, namely the changes of the duration, and of the strength of the premature ventricular contraction. It is well known that acceleration of the heart causes the aggregate of the periods of systole during 1 min. to become longer; but although the number of premature systoles is increased, each of them is shorter and weaker. In the case of considerable acceleration of the heart the weakening of the contractions may be so pronounced that the systemic output diminishes, while the inflow into the perfused artery becomes progressively less affected by the contractions. The ultimate result on the coronary blood flow is determined by the algebraic sum of these factors. Two extreme conditions of the heart can be imagined: the diastolic standstill with a heart rate of zero and a maximal coronary blood flow, and a sustained systolic contracture with a minimal coronary blood flow. Between these extremes should fall all possible heart rates. But under physiological conditions a prolonged systolic contracture cannot be obtained; with a rapid succession of premature beats the ventricles begin to beat so feebly that (considering the excessive heart rate from the point of view of the effect it has on the coronary circulation) it may be said that at very fast heart rates the heart approximates to a condition analogous to a diastolic rather than a systolic standstill. The weakening

of the contractions in the case of an increasingly rapid succession of premature contractions is especially marked in hearts which are not in good condition, as for example in cases of Ringer perfused hearts, or after administration of excess of carbon dioxide, or simply in hearts weakened by prolonged experimentation. In the whole animal a third factor has to be considered, namely changes in the aortic pressure. It has been shown in a previous communication (13) that the mean aortic pressure must be slightly raised in order to obtain a constant coronary blood flow in a heart-lung preparation in which the coronary arteries are left in connection with the aorta, and in which the heart rate is increased. In other words, in an ordinary heart-lung preparation the effect of acceleration of the heart rate is the same as in experiments with a perfused artery. But the effect of weakening the heart beat, which can be clearly observed in experiments with a perfused coronary artery, is not obvious in an ordinary heart-lung preparation. Ventricular fibrillation or the arrest of the heart leads to a complete removal of the resistance to the coronary blood flow on the part of the cardiac muscle itself. In the case of perfused arteries this leads to an augmentation of the coronary circulation. But when the coronary blood supply is dependent on the aortic blood-pressure and this falls to zero as the result of fibrillation, it is obvious that the coronary circulation must cease. Weakening of the contractions as the result of excessive acceleration of the heart also leads to a fall of the aortic pressure and hence the coronary blood flow declines, in spite of the diminished resistance which the heart opposes to it.

The following representative experiment illustrates the case of a strongly beating heart, each contraction of which (even at the fast rates) brought the coronary inflow to a standstill.

Heart-lung preparation. The circumflex artery was perfused at a pressure of 90 mm. of mercury. The mean aortic pressure was 110 mm. of mercury, the output 750 c.c. per minute, and the temperature 36° C. The S.A. node was cooled while the ventricle was driven at various rates. The figure marked with an asterisk is the spontaneous heart rate.

Heart rate per min.	75*	102	143	194	222
Coronary inflow in c.c. per min.	20·2	18·0	16·8	16·0	14·6

The hot-wire records of the coronary blood flow taken from a similar experiment are redrawn in Fig. 5. It can be seen at a glance that the areas of the curves become smaller as the heart rate increases. At the fastest heart rate the systolic restriction of the coronary inflow was not complete, but this weakening of the heart was insufficient to counterbalance the effect of the greater number of systoles per minute. In the

range of heart rates below about 100 beats per minute, the changes of the coronary blood flow are always more considerable; above the rate

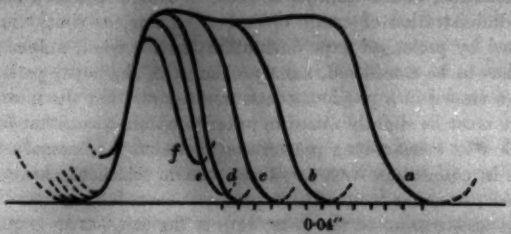

Fig. 5. Retraced corrected hot-wire records of the inflow of blood into the left coronary artery. The s.a. node was cooled during the whole experiment. Record (*a*) is taken during the spontaneous beats. Records (*b*) to (*f*) are taken during periods of driving the ventricle at various rates. Time 0·04 sec.

	Cycle in sec.	Coronary inflow per beat in c.c.	Heart rate per min.	Coronary inflow in c.c. per min.
(*a*)	0·96	0·308	62·5	19·2
(*b*)	0·78	0·207	83·5	17·8
(*c*)	0·56	0·143	107·0	15·4
(*d*)	0·42	0·103	143·0	14·7
(*e*)	0·35	0·084	171·5	14·4
(*f*)	0·28	0·063	207·0	13·9

of 100 the effect is small: in Fig. 5 a change of heart rate from 62 to 107 beats per minute diminished the blood flow by 3·8 c.c. per minute, while a change from 107 to 207 diminished it by only 1·5 c.c.

In hearts which are weak the diminution of the coronary blood flow with acceleration of the heart is much less conspicuous, and at faster rates it is often replaced by an augmentation. The mechanism of this effect is again revealed by the hot-wire analysis, an example of which is shown in Fig. 6. In this experiment the spontaneous heart rate varied between 123 and 128 beats per minute; the heart was not in good condition, as could be judged by the fact that, even at the beginning of the experiment, a rise of the aortic blood-pressure from 90 to 130 mm. of mercury led to a diminution of the systemic output from 750 c.c. to below 600 c.c. per minute, and the heart became unduly dilated. As the experiment continued the heart became weaker still; this could be clearly seen by the difference in the effect which acceleration of the heart had on the coronary circulation. Every 10 to 15 min. the ventricle was driven for periods of 2 to 3 min. at a rate of 182 per minute. A record of the coronary inflow was taken at the end of each period of acceleration.

Four such records are superimposed on one another in Fig. 6. It can be seen that during the first period of acceleration the inflow diminished

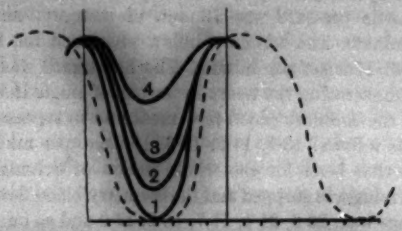

Fig. 6. Effect of acceleration of the heart beat on the inflow of blood into the left coronary artery. The dotted tracing is a record taken during the spontaneous heart beat. The tracings 1 to 4 were taken during periods of artificial acceleration of the heart. About 15 min. elapsed between each period of acceleration. Time 0·04 sec.

	Cycle in sec.	Coronary inflow per beat in c.c.	Heart rate per min.	Coronary inflow in c.c. per min.
Spontaneous rate	0·48	0·137	125	17·1
First period	0·32	0·077	182	14·0
Second period	0·32	0·094	182	17·1
Third period	0·32	0·101	182	18·4
Fourth period	0·32	0·130	182	23·7
Spontaneous rate	0·48	0·144	125	18·0

by 3·1 c.c. per minute, during the second period it remained constant, and during the third and fourth periods it increased by 1·3 and 6·6 c.c. respectively. Meanwhile the coronary inflow at the spontaneous heart rate remained practically constant. The cause of this difference in the effect of acceleration is shown by the corresponding hot-wire records. Each successive period of acceleration of the heart led to a progressively greater weakening of its contractions, and this was reflected on the coronary circulation, the effect of systole becoming smaller. A small dose of adrenaline (concentration about 1 in 200,000,000) was added before the fifth period of acceleration; the spontaneous heart rate increased by a few beats and the coronary blood flow remained unaffected. But during the fifth period of stimulation, when the heart was accelerated to 182 beats per minute, the coronary blood flow again (as in the first period) showed a diminution from 17·8 to 15·2 c.c.; hot-wire records showed that this was due to a greater compression (than in Period 4) of the coronary blood vessels, caused by the stronger beat.

The period of sudden transition from one heart rate to another is often accompanied by temporary changes of the coronary blood flow,

but these never last for more than about 30 sec. The following experiment illustrates the extent of these temporary fluctuations of the blood flow. The left coronary artery was perfused at a pressure of 70 mm. of mercury. The mean aortic pressure was 72 mm. of mercury, and the output 850 c.c. per minute. The coronary inflow proceeded for 15 min. at the rate of 16·6 to 17·0 c.c. per minute. The heart rate was 75 beats per minute. On accelerating the heart by means of single induction shocks to 155 beats, the coronary blood flow measured in successive periods of 10 sec. was as follows: 13·0, 14·2, 15·6, 15·2 c.c. per minute. The flow continued at that level for the whole period of driving; after about 10 min. the driving was stopped and the coronary blood flow in successive 10 sec. readings was: 23·6, 21·4, 17·7, 16·8, 17·0 and so on. The ultimate blood flow at 75 and at 155 beats per minute was 17 c.c. and 15 c.c. per minute respectively.

The effect of auricular fibrillation.

Miller, Smith and Graber(14) state that in some cases of auricular fibrillation the coronary blood flow was practically unaffected, while in others there was a slight augmentation or reduction. The rate of the coronary flow was usually increased in the experiments in which a rapid and more regular cardiac action followed the faradic stimulation of the auricles. In experiments on the whole animal in which the coronary perfusion pressure is that of the aorta, and in which the aortic pressure may vary as the result of irregularities of the heart beat, it is hardly possible to make deductions as to the direct effect of auricular fibrillation on the coronary circulation. Using the hot-wire method we performed a few experiments in order to determine whether auricular fibrillation had a direct effect. In order to keep the heart rate the same before and during auricular fibrillation we used (*a*) hearts in which complete A.V. block was produced by section of the bundle, and (*b*) hearts in which the ventricle was driven at a rate of 200 to 220 beats per minute, rates which are so fast that, at a temperature of about 36° C., they are not disturbed by the auricular fibrillation. In the first series of experiments the heart was driven with induction shocks which were applied in pairs, one shock to the auricle and the second after an interval varying between 0·15 and 0·22 sec. to the ventricle. These double shocks were applied at a rate above that of the spontaneous discharges from the S.A. node. In this manner the normal sequence of the contraction was imitated. In order to initiate auricular fibrillation a faradic current was sent through the auricular electrodes while the driving of the ventricle was continued.

Owing to the heart block auricular fibrillation had not the slightest effect on the ventricular heart rate. Similar experiments were also carried out without stimulation of the ventricle, and here the effect of auricular fibrillation was studied during idioventricular contractions. In the second series of experiments the bundle remained intact, and it was hoped that the rapid succession of premature ventricular contractions would not be disturbed by the onset of auricular fibrillation. In some of the experiments this was actually found to be the case.

Under the conditions just described we found that auricular fibrillation had no direct effect either on the magnitude of the coronary blood flow through the left artery, or on the character of the hot-wire record.

Conclusions.

1. Cooling and warming the blood perfusing a coronary artery increases and decreases respectively the blood flow through this artery. Registration of the rate of inflow of blood into the perfused coronary blood vessels by a hot-wire anemometer shows that the vessels dilate on cooling and contract on warming.

2. Cooling of the heart muscle, the heat of which is controlled, leads to a diminution of the coronary blood flow and warming to the reverse effect. The effect of cooling is due to a more forcible and more prolonged contraction of the heart at low temperature.

3. The direct effect of lowering the temperature on the coronary blood vessels, which favours the coronary circulation, is more pronounced than the indirect effect which is due to the changes in the strength of the ventricular contraction and which tends to diminish the coronary blood flow.

4. Auricular fibrillation has no direct influence on the coronary circulation when the rate of the ventricular contractions is controlled.

5. Ventricular fibrillation increases the blood flow through the perfused artery by removing the restricting effect of the systoles; this increase of the circulation is not due to the fibrillation actively assisting the propulsion of blood through the coronary system.

6. The effect of changes of the heart rate on the circulation through the perfused blood vessels is determined by the changes (*a*) of the duration, and (*b*) of the strength of the ventricular contractions.

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THE DETERMINATION OF THE CARBON DIOXIDE CONTENT OF THE MIXED VENOUS BLOOD.

Part II. Carbon dioxide equilibria between mixed venous blood and rebreathed airs.

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Since Henderson and Prince(13) first put forward their intermittent rebreathing method for the determination of the CO_2 tension of the mixed venous blood in the oxygenated state but still retaining its full complement of CO_2, several methods based on it as prototype have been published. In Table I are shown the variations of these methods.

They differ in (*a*) the composition of the gas mixture used; (*b*) the quantity of gas mixture; (*c*) the method of taking samples of the rebreathed air; (*d*) the method of carrying out the rebreathing; and (*e*) the length of the rebreathing period.

It is necessary to investigate in detail these variations in order to obtain a standard technique and to define the limits of application of the CO_2 data to the determination of the circulation rate.

With regard to the composition of the gas mixture, it has already been shown(15) that to ensure full oxygenation of the venous blood a very high initial percentage of oxygen is required. The mixture used by the writers contained 93·5 p.c. oxygen and 6·5 p.c. CO_2, the CO_2 being added to render more rapid the attainment of equilibrium.

Whether the quantity of gas mixture is critical depends firstly on the method of sampling used, and secondly on the necessity for having sufficient oxygen finally present in the rebreathing bag to maintain the oxygen tension above the critical level (see (15)).

If samples are taken from the rebreathing bag, it is essential that the whole of the air in the lung-bag system should be of uniform composition; the volume of gas should therefore not be large. The question has been investigated by Lundsgaard and Schierbeck(18) and by Grollman and Marshall(9). The latter authors consider that 2·5 litres is a correct volume. If, however, the sample is taken from the last of

the air expired from the lungs into the rebreathing bag, the sample being taken as near to the mouthpiece as possible, complete homogeneity of the lung-bag mixture is no longer of first importance; it is only required that the gases in the depths of the lungs should have become equilibrated. The quantity of gas mixture is therefore not so crucial, and this consideration makes this method of sampling much superior to the former method.

As far as the second point is concerned, we had already found (15) the use of 4 litres of the gas mixture given above to be satisfactory and to permit of rebreathing periods of sufficient length without too great a fall in the oxygen pressure.

TABLE I.

Authors	Rebreathing period (sec.)	Number of respirations per rebreathing period*	Volume of gas used (litres)	Method of sampling
Henderson and Prince (11, 13)	5–15	Breath held	1·0–1·5	From bag
Meakins and Davies (20)	10–15	2	4·5–5·0	Last expired air
Field, Bock, etc. (8)	15	4–5	4·5–5·0	From bag
Brocklehurst, Henderson and Haggard (4)	15–30	1 every 5 sec.	6·0–7·0	Last expired air
Bock, Dill and Talbott (3)	12–16	Breath held	4·5–5·0	Last expired air

* By "rebreathing period" is meant the time between the first inspiration from the rebreathing bag and the beginning of the final expiration into the bag.

With regard to the method of rebreathing, it will be seen that the breath may be held in the lungs throughout the rebreathing period, or the subject may carry out a number of respirations during the period; *i.e.* the gas in the rebreathing bag is breathed to and from the lungs two or more times, the bag being more or less completely emptied at each respiration. The object of this procedure is to avoid any hindrance to the circulation that holding the breath might cause (Laurens (17), Douglas and Haldane (6)), and for other reasons (see below).

The rebreathing time is usually assessed on the assumption that the circulation time in man at rest is about 20 sec. Vierordt (23) found that in mammals of widely different sizes the circulation time was between 26 and 28 heart beats; Y. Henderson (12) considers this the most accurate estimate available, and it might be used for estimating the circulation time in different subjects. Blumgart and Weiss (1) using a radium method give 14 to 24 sec. as an estimate. The proper assessment of the rebreathing time is however bound up in a fundamental assumption of all Henderson-Prince type methods, an assumption that has been somewhat neglected. It may be explained as follows.

The rise of CO_2 tension in an oxygen and CO_2 mixture with successive

periods of rebreathing becomes rapidly less until a plateau level is ultimately reached. This level, which may be designated the "primary plateau level," is, as was first shown by Henderson and Prince(13), taken to indicate the CO_2 tension of the mixed venous blood. The primary plateau level however does not at all times indicate this tension. Whether the primary plateau level is, or is not, a true one depends on the length of the rebreathing period. False plateaus occur when the rebreathing time falls outside certain limits. If the primary plateau levels are plotted against rebreathing time, a curve is obtained as shown in Fig. 1. It is only for rebreathing periods falling within the times covered by the flat part of the curve, the "secondary plateau," that the primary plateau levels truly indicate the CO_2 tension of the oxygenated venous blood.

The appearance of the secondary plateau is due to two sets of factors. The period of approach to this plateau, marked T_1 in Fig. 1, is due,

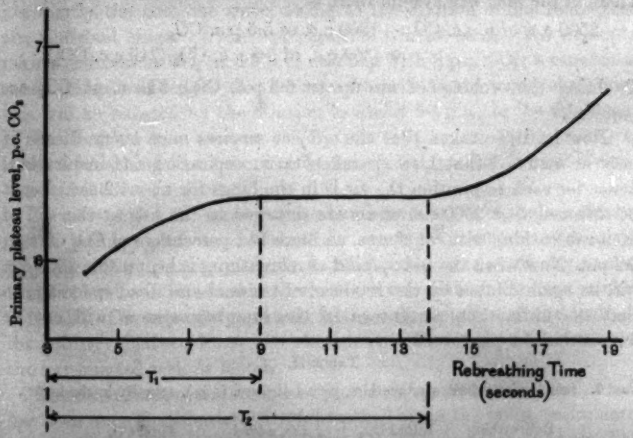

Fig. 1. The relation between primary plateau level and the length of the rebreathing period, to show the formation of a "secondary plateau" with limits T_1 and T_2.

firstly, to the fact that when a gas mixture is taken into the lungs with a CO_2 tension differing more or less from that of the mixed venous blood, a certain time must elapse while the two come into equilibrium by diffusion of CO_2 one way or the other. This period will be appreciable because of the large volume of gas in the lungs, and because the rate of

diffusion of CO_2 through the lung membrane, though rapid, is still sufficiently slow to make its effect felt. And secondly, as pointed out by Hamilton, Moore and Kinsman(10), it is due to time taken up in replacing any arterial blood left in the lungs by venous blood.

The effect of the first of these factors is exemplified by the following calculation.

Let the CO_2 output = 220 c.c. per min. = 3·7 c.c. per sec. Suppose that there are 2·5 litres of a 6 p.c. CO_2 gas mixture in the bag. Let the alveolar CO_2 be 5·5 p.c. and the venous CO_2 be 6·5 p.c. When the bag contents are taken into the lungs they will be diluted by (i) the residual air, (ii) the dead-space of the subject under the prevailing conditions, (iii) the dead-space of the apparatus and mouth-piece. As a deep expiration to air is made preliminary to the experiment, these may be assumed to be filled with alveolar air.

Let the residual air = 1500 c.c. and the total dead-spaces = 300 c.c. Then, in the lung-bag system there is

2500 c.c. of 6 p.c. CO_2 + 1800 c.c. of 5·5 p.c. CO_2
= 4300 c.c. of 5·8 p.c. CO_2 (249 c.c. CO_2).

To bring this volume of air up to 6·5 p.c. CO_2, 31 c.c. of CO_2 are required.

Now let it be taken that the subject respires once every 3 sec.; it may be assumed that 1 sec. is taken up in expiration and inspiration; hence for each respiration the air is in the lungs for about 2 sec. Let it be assumed that 2500 c.c. of air are returned to the bag at the end of each rebreathing with, of course, an increased percentage of CO_2 of, say, 6·2 p.c. Now when the next period of rebreathing is begun this 2500 c.c. will be again diluted by the 1800 c.c. of residual and dead-space air, so that the initial CO_2 percentage in the lung-bag system will not be 6·2 p.c. but 5·9 p.c.

TABLE II.

Case 1. Two respirations in a rebreathing period of 6 sec. (4 sec. actually in the lungs).

Rebreathing number	Initial CO_2 p.c.	Amount of CO_2 added c.c.	Final CO_2 p.c.
1	5·80	14·8	6·13
2	5·87	14·8	6·21
3	5·91	14·8	6·26
4	5·94	14·8	6·28
5	5·95	14·8	6·30
6	5·96	14·8	6·31

Case 2. Three respirations in a rebreathing period of 9 sec. (6 sec. actually in the lungs).

1	5·80	22·2	6·31
2	5·97	22·2	6·49

In Table II is shown how, in these theoretical conditions, the CO_2 content and pressure (in percentage of one atmosphere) in the rebreathing bag vary with the number of rebreathings.

The rate of CO_2 diffusion has been taken to remain constant, and this seems to be justifiable, except in the very last stages, if Kerridge's estimate(16), that a pressure difference of 0·016 p.c. of an atmosphere is sufficient to maintain the resting CO_2 diffusion rate, be accepted.

The results obtained in Case 1 for the fifth and sixth rebreathings would be taken as evidence that a plateau had been reached, but it is 0·2 p.c. CO_2 below the true level. Such an error would cause the circulation rate to be over-estimated about 20 p.c. (see (15)).

The calculation also shows, as pointed out by Hamilton, Moore and Kinsman(10), that although the same plateau may be reached whether the initial CO_2 pressure in the rebreathing bag is greater or less than the CO_2 tension of the mixed venous blood, this plateau is not necessarily the true one since, owing to the dilution of the rebreathed air, the final stages of the equilibration are the same. For example, taking the venous CO_2 as 6·5 p.c., starting with 8 p.c. CO_2, a number of rebreathings will reduce this to, say, 6·6 p.c. At the next rebreathing this will be reduced by the dilution to about 6·4 p.c., so the final stage of equilibration is an *increase* of CO_2 from 6·4 p.c. to 6·5 p.c., exactly as if the rebreathing had been started from a CO_2 p.c. lower than that in venous blood.

In view of this the use, by several authors (3, 13, 20), of the coincidence of the primary plateaus, obtained by using gas mixtures with initial CO_2 tensions respectively above and below the tension of oxygenated venous blood, as a criterion as to whether a true plateau has been reached is invalid. The secondary plateau is the only criterion of the truth of the primary plateau level, *i.e.* whether it indicates the CO_2 tension of the oxygenated venous blood.

The other limit of the secondary plateau is reached when blood, which has been once equilibrated with rebreathed air in the lungs, recirculates in sufficient amount to raise the CO_2 tension in the rebreathed air. This time is marked T_2 in Fig. 1.

It will be seen that if T_1 is equal to or greater than T_2, or if the secondary plateau be so circumscribed as to be practically absent, the method is useless, as it is impossible to tell whether or not the primary plateau level truly represents the CO_2 tension of the oxygenated venous blood.

These methods have been attacked at this admittedly vulnerable

point by Hamilton, Moore and Kinsman(10). They state that it is not possible to demonstrate what has here been designated the secondary plateau, and in fact, that the curve of primary plateau level against rebreathing time shows a steady rise.

Their explanation for this is that the period required for the complete replacement of the arterial blood in the lungs by venous is so long that, in the nomenclature used here, T_1 becomes equal to or greater than T_2. In support of this they quote evidence by Drinker and others(7) that at rest only the easiest routes transmit blood, which is moving sluggishly along other routes, *i.e.* there is a "tailing" of blood flow through the lungs.

Now no other authors had noticed this absence of the secondary plateau. Bock, Dill and Talbott(3) thought that a "secondary plateau" existed, at rest, over the range 12 to 16 sec. Y. Henderson(4, 11, 13) has ascribed more extended limits to this plateau. With regard to the tailing of blood through the lungs, Blumgart and Weiss(2) consider they have evidence which indicates that it is of no importance in normal subjects, and Meakins and Davies(21) are of the same opinion.

This was not the only reason why we questioned the results of Hamilton, Moore and Kinsman. They carried out a series of determinations of the arterio-venous CO_2 pressure difference using a method, for the venous CO_2, very similar to that advocated here, and using rebreathing times varying from 6 to 15 sec. Their results show the absence of a secondary plateau. They claim that their results show a "probable error" of $\pm$ 0·12 mm. Hg, *i.e.* 0·016 p.c. atmosphere, on the average, in the determination of the arterio-venous CO_2 pressure difference. It is not stated what is meant by probable error, but it may be assumed that the statistical definition of the term is implied. This, unfortunately, gives only an imperfect indication of the accuracy of the results, but it can be calculated that the standard deviation of their results would be about $\pm$ 0·024 p.c. In the opinion of the writers this represents an improbable degree of accuracy. Henderson and Bock, Dill and Talbott (*loc. cit.*) average figures differing by 0·1 p.c. CO_2 or more to obtain their primary plateau levels. But these limits, on the other hand, might be thought too wide.

Some experiments were therefore carried out to obtain an idea of the possible accuracy of the method in use. The tension of the CO_2 of the oxygenated venous blood was estimated by exactly the same procedure five times consecutively at one sitting. Such a series takes about 1½ hours to complete, exclusive of a preliminary rest period of ½ hour. The results

from the two best experiments show standard deviations of ± 0·04 p.c. CO_2 for the subject M. C. G. I. and ± 0·06 p.c. for the subject F. W. L. Both subjects have considerable experience of respiratory methods, but the former subject had had more practice at this particular method. Evidently it does not seem reasonable to expect an accuracy of much greater than ± 0·08 p.c. CO_2 for the ordinary routine of experiments such as these.

In the experiments of Hamilton, Moore and Kinsman two experimental conditions were being simultaneously varied, viz. rebreathing time and the number of respirations per rebreathing period. For in their experiments the subject respired once every 3 sec., so that if the rebreathing period were 6 sec., there were two respirations per rebreathing period; if 9 sec., three, and so on. Now it has been shown by Laurens (17), by Hill and Flack (14) and by Wardlaw (24) that respiratory movements may affect the output of CO_2 from the blood. Consequently this possibility had to be taken into account.

Four series of experiments were therefore carried out by the writers.

(1) Repetitions of Hamilton, Moore and Kinsman's experiments, as described in their paper.

(2) Experiments with varying rebreathing times, but with the number of respirations per period constant.

(3) Experiments with varying numbers of respirations in rebreathing periods of the same length.

TABLE III.

First Series. Primary plateau level. CO_2 p.c. atmosphere. Subject: M. C. G. I.

Date	19. ix. 28	21. ix. 28	22. ix. 28	25. ix. 28
2 respirations in 6 sec.	6·56	6·76	6·43	—
3 respirations in 9 sec.	6·54	6·73	6·78	6·73
4 respirations in 12 sec.	6·53	6·77	6·79	6·86
5 respirations in 15 sec.	—	—	—	6·96

Second Series. Primary plateau level. CO_2 p.c. atmosphere. Subject: M. C. G. I.

Date	27. ix. 28	2. x. 28	2. x. 28	3. x. 28
3 respirations in 9 sec.	6·80	6·76	6·78	7·02
3 respirations in 12 sec.	6·82	6·74	6·85	7·04
3 respirations in 15 sec.	6·94	6·90	6·88	7·06
Date	4. x. 28	4. x. 28	5. x 28	5. x. 28
2 respirations in 8 sec.	6·98	6·96	6·92	6·72
2 respirations in 10 sec.	6·98	7·03	6·93	6·78
2 respirations in 12 sec.	6·96	7·04	6·97	6·73

Third Series. Primary plateau level. CO_2 p.c. atmosphere. Subject: M. C. G. I.

Date	9. x. 28	10. x. 28	12. x. 28	23. i. 29
2 respirations in 12 sec.	7·02	6·76	6·84	7·07
3 respirations in 12 sec.	6·79	6·80	6·89	7·04
4 respirations in 12 sec.	6·80	7·00	6·96	—
Breath held for 12 sec.	—	—	—	7·07

Representative results of these experiments are given in Table III. They show, in the first series, that Hamilton, Moore and Kinsman's results could not be satisfactorily confirmed. In the second series the presence of a secondary plateau is better marked. The third series seems to indicate that with the subject used, a rate of respiration of once every 3 sec. is apt to cause an increase in the venous CO_2 tension, especially when there are more than three respirations per period; but rates lower than this have no effect.

(4) Observations from the preceding series of experiments lead the writers to believe that there is an optimum rate of respiration for procedures of this type, which varies from subject to subject. In this series of experiments the subject was allowed to use the rate of respiration most comfortable for him (or her); the subject was not required to make definite respirations during the rebreathing period, but merely to respire gently into and out of the bag, without necessarily emptying it, after the first inspiration. Such a rate usually worked out at about one respiration every 4 sec.

Typical results of these experiments are shown in Table IV.

TABLE IV.

Primary plateau level. CO_2 p.c. atmosphere.

Subject	M.C.G.I.	F.W.L.	F.W.L.	E.D.	E.M.J.	E.M.J.	B.F.	G.V.W.	R.V.W
Date	3. i. 29	5. ii. 29	7. ii. 29	6. ii. 29	26. ii. 29	5. iii. 29	27. ii. 29	28. ii. 29	14. iii. 29
Rebreathing time:									
8 sec.	6·79	6·31	6·43	7·40	6·30	6·46	7·31	6·12	6·15
10 „	6·84	6·48	6·47	7·31	6·66	6·43	7·29	6·13	6·20
12 „	6·80	6·47	6·40	7·26	6·69	6·54	7·37	6·30	6·23
14 „	7·01	6·50	6·49	7·43	6·84	6·50	7·41	6·31	6·20

The results of over 40 experiments on 7 subjects convince the writers that the secondary plateau does in fact exist, but also, that its limits are more circumscribed than has formerly been thought. The safety limits for the secondary plateau are 10 to 12 sec. (inclusively), although in some of the subjects examined it was found to be more extensive.

Hamilton, Moore and Kinsman also attempted to estimate the total CO_2 in the lung-bag system at various times during the rebreathing period. They claim to have shown that about 13 c.c. of CO_2 passes from the lungs into the blood during the first part of the rebreathing period. But in view of the fact that the most accurate methods of estimating the residual air in the lungs (19, 22) do not claim to give results nearer than 100 c.c., and that Christiansen, Douglas and Haldane (5) abandoned an attempt to determine the total amount of CO_2 in the lungs owing to

the impossibility of estimating the amount that went into solution and combination in the lung tissues, it is difficult to place much reliance on Hamilton, Moore and Kinsman's results. It has consequently not been thought worth while to repeat these experiments.

In conclusion it may be said that it is safe, with normal subjects in the resting state, to assume the presence of a secondary plateau over the range 10 to 12 sec. The rebreathing period in experiments to determine the CO_2 tension of the oxygenated mixed venous blood should therefore be carefully timed to fall within this range; especially if the result is to be applied to the determination of the circulation rate, owing to the exaggeration of errors as shown in our previous paper (15).

In exercise both diffusion rate and circulation time are changed, so that the secondary plateau limits will be shifted in such a way that the time limits become less, as has been shown by the results of Bock, Dill and Talbott (3).

For the greatest accuracy it would be necessary to establish the limits of the secondary plateau for any given subject under conditions of exercise or disease.

Summary.

1. An investigation of the various proposed modifications of the intermittent rebreathing method for the determination of the CO_2 tension of the oxygenated venous blood has been made. As a result of this it is proposed that the following details of technique should be adopted:

(*a*) The rebreathing bag should be filled with 4 litres of a mixture of 93·5 p.c. oxygen and 6·5 p.c. CO_2.

(*b*) Samples should be taken from the last of the air expired into the rebreathing bag from a deep expiration at the end of a rebreathing period.

(*c*) The subject should respire to and from the bag at a slow rate, best suited to himself, during the rebreathing period.

(*d*) The length of the rebreathing period should be accurately timed to fall on the range covered by the secondary plateau. This is from 10 to 12 sec. in the normal resting subject.

2. The existence of a "secondary plateau" in the curve relating primary plateau level to rebreathing time is demonstrated.

3. The fact that the rebreathing period falls within the range covered by the secondary plateau is the only criterion as to whether or not the primary plateau level truly represents the CO_2 tension of the oxygenated venous blood.

4. Consequently the secondary plateau should be delimited if the conditions are such that a change in its limits are to be expected, such as at exercise or in pathological conditions.

The writers' thanks are due to those members of the staff and students who acted as subjects for these experiments.

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THE DEPOLARIZATION OF CRUSTACEAN NERVE BY STIMULATION OR OXYGEN WANT.

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In 1927, at the time of his sudden untimely death, Levin, working with crustacean nerve, was investigating a remarkable characteristic of the action current, which he termed the "retention of action current," and the extreme fatigability of the nerve in question (1). His chief results may be summarized as follows:

1. The galvanometer deflection (the "negative variation of the injury current") due to a continued tetanus through a single pair of stimulating electrodes increased with the time, reached a maximum in about 20 sec., and then gradually declined. The galvanometer leads were monophasic, non-polarizable electrodes were used, and the nerve was in moist air.

2. If stimulation were stopped within two minutes the galvanometer moved quickly back to a certain position and then returned only slowly towards its original place. The size of the rapid swing back decreased as the duration of the stimulus increased: no back swing at all was observed after two minutes of continuous tetanization. The time required for the galvanometer to return to its original position after the end of a stimulus depended upon the duration of that stimulus, being from 1 to 10 min. or more. Levin described this phase of slow return by the term "retention of action current."

3. The maximum displacement of the galvanometer from its earliest unstimulated position was the same in a succession of tetanic stimuli (the "ceiling effect"), whatever the position of the galvanometer at the beginning of any particular stimulus, provided that the nerve was not over-stimulated by too strong a faradic current, or unduly fatigued by too long a stimulus. In other words, the sum of (*a*) the "retention," and (*b*) the action current produced by a stimulus, was constant.

4. Fatigue set in particularly quickly in the neighbourhood of the stimulating electrodes.

Levin suggested that the disappearance of his "retention" was due to some kind of restitution process following activity.

At the beginning of 1928 Prof. A. V. Hill suggested to me that I should further investigate these remarkable properties of crustacean nerve. The present work, therefore, was undertaken at the Marine Biological Laboratory at Plymouth and was continued through the year. A brief account of the earlier experiments was communicated to the Physiological Society (2).

Method. The method employed was nearly the same as Levin's, with a few minor alterations from time to time. A high resistance sensitive moving-coil galvanometer of long period (Campbell galvanometer by the Cambridge Instrument Company) was at first employed, as the moisture of the sea air had injured the moving magnet instrument employed by Levin. The disadvantage of the Campbell galvanometer for this type of experiment is its very long period (25 to 30 sec.), and at the end of March it was changed for a sensitive and rapid moving-magnet instrument constructed and erected by Mr A. C. Downing. This galvanometer with four high resistance coils (20,000 ohms in series) can be adjusted to as high a sensitivity as 1 mm. $= 5 \times 10^{-13}$ amp. on a scale two metres distant, with a deflection time of about 2 sec. when critically damped. Such a sensitivity, however, is not required except when a single impulse only is investigated. Usually a much lower sensitivity was employed, the galvanometer being critically damped with a deflection time of about 1 sec. The preparation of the nerve was modified from Levin's practice. The leg (usually of a spider crab, but sometimes the claw of a lobster or the leg or claw of an edible crab) was severed near the body, the two junctions between the third and fourth segments were cut by bone forceps, and the muscles attached to the fourth segment were cut away. The two portions thus made were then gently pulled apart, the nerve remaining attached to the fourth segment. The whole process of "pulling out" the nerve may take less than half a minute. The nerve so obtained was washed immediately in sea-water. Lastly, the silver stimulating electrodes were replaced by narrow strips of filter paper soaked with sea-water. These paper electrodes were connected to the copper leads from the secondary of a Harvard stimulating coil (about 50 make and 50 break shocks per second) through holes bored in the block of wax constituting the nerve chamber, the holes being filled with sea-water gelatin. Thus the spread of copper ions into the stimulating electrodes was prevented. The chief reason for this change is to avoid the damage caused by silver wire to the nerve which is much more liable to mechanical injury than a medullated nerve.

Repetition of some of Levin's experiments. Some of Levin's experi-

ments were repeated, employing the moving-coil galvanometer. The deflection during the period of "retention" was recorded every half minute. The deflection ("negative variation") produced by tetanic stimulation increases at first with continuance of the stimulus, passes through a maximum, and then declines gradually. When stimulation ceases before three minutes there is a rapid back swing, the amount of which depends upon the duration of the stimulus: it vanishes after about three minutes' continuous stimulation. The back swing is followed by a long period during which the "retention" gradually disappears and the galvanometer slowly returns to its original position. The complete disappearance of "retention" is achieved within 17 to 50 min., according to the period of stimulus given (10 to 37 sec.). In the present experiments the periods of "retention" are more exaggerated than those observed by Levin. They cannot of course be measured very accurately as the galvanometer returns asymptotically to its final position. The duration of the stimulus in a number of experiments, the maximum deflection, and the period of "retention" are given in Table I.

TABLE I. Periods of action current "retention" in air.

Date (1928)	29. i.	29. i.	29. i.	30. i.	30. i.	30. i.	15. ii.	16. ii.	13. iii.	13. iii.
Experiment	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)			(*a*)	(*b*)
Duration of stimulation (sec.)	17	18	20	32	35	26	10	37	35	31
Maximum deflection (mm.)	422	430	387	287	830	481	278	850	181	535
Period of retention (min.)	23	30	32	21	30	30	17	50	43	22

From Levin's work it is clear that the magnitude of the deflection, caused by a second tetanic stimulus given at an interval after a first, is determined by the amount of the first action current still "retained" at the moment. The second response, therefore, can be fully displayed only when the first "retention" has disappeared completely. Fig. 1 shows the time curve of the height of the second deflection. Two stimuli were given at an appropriate interval. In order to avoid unnecessary fatigue at the stimulating electrodes the stimulus was cut off as soon as the deflection had reached a maximum. At the desired interval after the first stimulus the potential divider which supplied current to balance the injury potential was adjusted so as to bring the galvanometer to zero, and the second stimulus was applied. The ratio of the two deflections thus obtained is given in Fig. 1. An ample interval (30 to 50 min.) was allowed between two successive pairs of observations. The percentage recovery of the second deflection, beginning at a low value immediately

after the first stimulus, attains 50 p.c. in 2 to 4 min., then increases steadily, and at about 15 min. may reach 80 p.c. or more. These experiments, and all subsequent ones unless otherwise stated, were made in air.

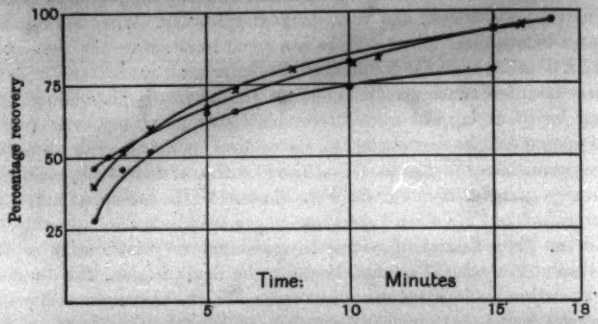

Fig. 1. Size of action current ("negative variation") in response to a second tetanus at various intervals after a first one: second response expressed as a percentage of first: three separate experiments: nerve in air.

The results show clearly that, in order to obtain the full value of a second deflection, the "retention" of the first action current must be completely eliminated, and that in the "retention" phenomenon we are concerned with some restitution process, as Levin suggested.

Injury potential and maximal "negative variation." During the earlier experiments a peculiar relation was noticed between the maximum deflection due to a tetanic stimulus given by a single pair of electrodes, and the injury potential existing at the moment. The two quantities seem to be connected in a simple manner. Altogether nineteen sets of observations were made during February, 1928, of which typical examples are shown in Fig. 2. The magnitude of the injury potential is a function of the time from the moment of preparation of the nerve. It increases often during a short period after the nerve is first prepared, then decreases rapidly for 30 min. or so, and then very slowly, at an approximately constant rate, until finally it disappears. The maximum deflection obtained on tetanizing follows the injury potential in an approximately linear manner, and tends also to disappear when the injury potential approaches zero. The periods of observation extended from 2 to 23 hours. Such a remarkable relation could hardly be expected unless we assume that the injury current and the action current are in some way related.

At present, however, in the nerves or other tissues of the various animals studied, there is little evidence suggesting any such relation. In the case

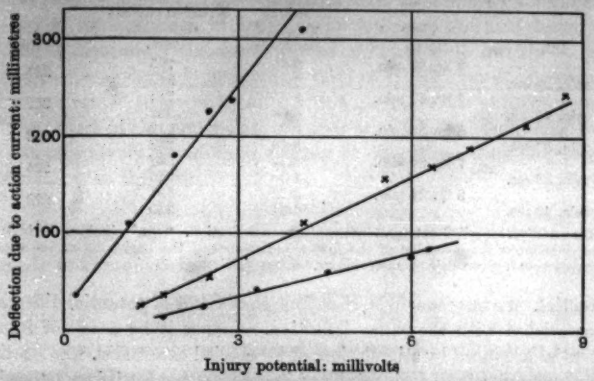

Fig. 2. Deflection due to action current, as a function of the magnitude of the injury potential existing at the moment: injury potential diminishing spontaneously with time: three separate experiments: nerve in air.

of frog's nerve, for example, it has frequently been observed that even when the injury potential has almost completely vanished the action current remains approximately unchanged.

The injury potential can be diminished not only by time but by stimulation. Take the case of two tetanic stimuli at an appropriate interval: denote the first maximal deflection by d_0 and the injury potential before stimulation by p_0. After a short interval (1 to 2 min.) from the first stimulus, adjust the potential divider to a value p_1 to bring the galvanometer deflection to zero. Apply a second stimulus, resulting in a maximum deflection d_1. The ratio $\frac{d_0 - d_1}{p_0 - p_1}$ gives us the change of action current (say in mm. deflection) associated with a change of one unit of injury potential (say 1 millivolt). This quantity is then compared with the deflection caused by one unit of potential applied externally, by means of the potential divider, through the galvanometer and nerve. Seven experiments of this nature were performed during February, 1928, with satisfactory results. One of these is described in Table II.

The agreement between the two values found is remarkable, the mean value of the ratio being 220 mm. per millivolt, while 221 mm. was obtained by the external application of the same difference of

TABLE II. Change of action current per unit change of injury potential produced by stimulation.

Int.	Time p.m.	Injury potential millivolts	Action current deflection mm.	$\frac{d_2 - d_1}{P_2 - P_1}$
	3 hr. 50 min.	9·51	795	221
1 min. 39 sec.		7·34	315	
	4 hr. 47 min.	8·55	725	222
1 min. 40 sec.		6·60	292	
	6 hr. 32 min.	6·98	618	213
1 min. 37 sec.		5·29	258	
	7 hr. 36 min.	6·23	567	225
1 min. 39 sec.		4·80	245	
	8 hr. 31 min.	5·72	537	222
1 min. 43 sec.		4·34	230	

One millivolt applied externally gave 221 mm. deflection. "Int." signifies the interval allowed between the cessation of the first stimulation and the beginning of the second stimulation in a given pair of observations.

potential. We may conclude, therefore, that a unit of potential difference is associated with the same deflection, whether it be a fall of injury potential produced by stimulation, or be applied externally. The relation found can scarcely be accidental, and it suggests that the injury potential, or whatever it implies, is in a sense, in crustacean nerve, the cause of the action current, just as an applied electromotive force is the cause of the resulting deflection.

The temporary depolarization of nerve. We have just shown that the ratio

$$\frac{\text{(decrement in the response to stimulation)}}{\text{(decrement in injury potential)}},$$

both decrements being the result of a previous stimulus, is equal to

$$\frac{\text{(deflection due to an applied E.M.F.)}}{\text{(E.M.F. applied)}}.$$

It might have been expected that each would be equal to

$$\frac{\text{(total response to stimulation)}}{\text{(total injury potential)}}.$$

This expectation is not verified. The ratio last named, with a single pair of stimulating electrodes, is usually much less than the others. The discrepancy might be accounted for if the strength of the stimuli were not sufficient to stimulate all the nerve fibres, or if local fatigue at the stimulating electrodes were so profound as to prevent further activity in the nerve trunk. The latter seems the more probable, but the former cannot be neglected, as was found in later experiments with the Downing galvanometer, when for other purposes a single impulse was recorded. If the second suggestion were correct local fatigue could be avoided by stimulating a fresh point on the nerve when signs of fatigue had set in

at the first electrodes. Accordingly a wax chamber with three pairs of stimulating electrodes was made. Three pairs were found to be sufficient for the present purpose. These electrodes were used in such a way that as soon as the deflection caused by stimulating at the first electrodes reached a maximum, the stimulus was switched over quickly to the second pair, and similarly later to the third pair. This type of experiment can be performed successfully only with a galvanometer of short period.

The object of the present experiments is as follows. Supposing that we can effectively avoid local fatigue at the stimulating electrodes, how closely can the ratio

$$\frac{\text{(total response to stimulation)}}{\text{(total injury potential)}}$$

be made to approximate to the ratio

$$\frac{\text{(decrement in response to stimulation)}}{\text{(decrement in injury potential)}}?$$

The question can really be expressed as follows: How far can we abolish the injury potential during, and by, stimulation if we avoid appreciable local fatigue? In the following experiments the injury potential was not balanced. The sensitivity of the galvanometer was suitably reduced and the deflection on the scale was a direct consequence of the injury potential. The electrical response to stimulation diminished the deflection. Our problem, therefore, was, how closely can the galvanometer be brought to zero during, and as the result of, stimulation?

Certain precautions necessary for this type of experiment must be described. Firstly, the injury must be complete, otherwise an apparent reversal of the injury potential may be obtained as the result of stimulation, as the following experiments show:

Injury potential (millivolts)	9·68	7·70	8·72
Deflection due to injury potential (mm.)	225	210	222
Residual deflection after stimulating (mm.)	−90	−80	−38

On some occasions a nerve trunk was found to have opaque patches (the normal nerve is quite transparent and has a very faint bluish tint) which block conduction. In one of these cases the two galvanometer leads were placed at intact points on either side of an opaque region. There was only a small potential difference (0·92 millivolt) between the two leads, which may be attributed to a difference of the nerve's resting activity at the two points. On stimulation, the maximum deflection (one pair of stimulation electrodes used) corresponded to 2·57 millivolts. This means that if the resting activity were equal on both sides of a block, we might record action current without injury potential. An incomplete injury may act as a partial block and leave some of the partially injured fibres inactive, though not exhibiting an injury potential. This may explain the apparent reversal, under such circumstances, of the injury potential as the result of stimulation.

Secondly, the strength of the stimulating current must be just, and only just, sufficient to give a maximal effect. Over-stimulating induces rapid local fatigue, or may inflict damage on the nerve around the stimulating electrodes, even though the stimuli used be no stronger than those employed for frog's nerve. The procedure adopted was to use at first a weak stimulus and then gradually to increase its strength in successive observations until finally a maximum degree of depolarization was obtained. An example will show the importance of not employing too strong a stimulus.

Time	Injury potential millivolts	Deflection due to injury potential mm.	Coil distance cm.	Residual deflection during stimulation mm.
11.57 a.m.	5·57	284	9	10
1.30 p.m.	5·23	260	8	33
3.30 „	5·18	275	7	90
4.25 „	5·06	319	5	160

The residual deflection is seen to *increase* with stronger stimulation instead of decreasing. Probably some of the fibres were put out of action by the strong current employed. The third, and the most important, factor for a successful experiment is the condition of the nerve itself. Uninjured points of this should be at, or approximately at, the same potential, and the excitability should be constant. These conditions are not always readily attained. To attain them requires care in the preparation of the nerve. It is often found that excitability varies considerably along the length of the nerve. Another important source of error lies in the onset of the type of inexcitability described below. In spite, however, of these difficulties, it is possible, on occasion, to obtain decisive results.

A series of preliminary experiments was made with the slow-moving Campbell galvanometer, the results of which are given in Table III.

TABLE III. Diminution of injury potential by stimulation: slow-moving galvanometer.

Date (1928)	6. iii.	7. iv.	8. iv.	9. iv.	10. iv.	11. iv.	13. iv.
Initial injury potential (millivolts)	4·23	6·88	2·59	4·04	2·54	3·21	5·29
Residual injury potential at end of stimulus (millivolts)	1·27	3·03	0·01	0·49	0·88	0·37	0·08

As mentioned above, this type of galvanometer is unsuitable for the present experiments. It can, nevertheless, be seen that the injury potential falls temporarily to a small fraction of its original value after a short tetanic stimulation at several pairs of electrodes. After this preliminary success the experiments were continued with the Downing galvanometer. Owing to its short deflection time (about 1 sec.) it was

extremely satisfactory. It was suitably shunted in order to keep the spot of light within a convenient range on the scale. The total time of stimulation was reduced to 5 to 10 sec. instead of the 20 to 30 sec. required with the Campbell galvanometer. The delay in attaining the maximum with the Downing instrument—5 to 10 sec.—is a genuine effect to be attributed to the properties of the nerve; the deflection time of the galvanometer itself is much less. The results of experiments with the Downing galvanometer are given in Table IV.

TABLE IV. Temporary total depolarization by stimulation: rapid galvanometer.

Date (1928):											
5. iv. (*a*)	5. iv. (*b*)	12. iv.	14. iv.	16. iv.	20. iv.	23. iv.	24. iv.	25. iv.	28. iv.	3. v.	4. v.
Injury potential (millivolts):											
2·61	3·90	6·73	1·14	12·80	2·08	10·87	5·57	7·99	5·04	10·12	6·99
Deflection due to injury potential (mm.):											
64	86	303	410	212	312	249	284	262	85	250	160
Residual deflection (mm.):											
1	20	0	0	0	4	0	10	0	0	5	0
Percentage abolition:											
99	77	100	100	100	98	100	96	100	100	98	100

The residual deflection given in the fourth row can be maintained only for a short period as fatigue at the stimulating electrodes causes a quick return of the spot of light towards its original position. It must be stated that the results given in Table IV are the best performances in a given set of observations. Several observations were generally made before the result shown was obtained (compare the second precaution discussed above). The injury potentials observed in these cases are higher than those of Table III. In seven cases out of twelve the deflection due to the injury potential was completely abolished, that is to say, the galvanometer had returned precisely to its zero at the moment when the "negative variation" had reached its maximum. In these experiments there was no over-shooting of the deflection beyond zero. It might be suggested that the absence of over-shoot was accidental and that the "negative variation" might more than abolish the injury current. The following example shows how, in successive observations over a considerable interval, the maximum response to stimulation may continue exactly to abolish the injury current deflection.

Time	Injury potential millivolts	Deflection due to injury potential mm.	Residual deflection mm.
2.55 p.m.	12·80	212	0
3.50 „	11·52	203	0
5.10 „	9·94	182	0

The first result of this experiment has already been recorded in Table IV (April 16). Two more observations were made later. The residual deflections remained nil. The last observation was made two and a quarter hours after the first one, when the injury potential had fallen by about 3 millivolts. It would be difficult, therefore, to attribute the equality of the two deflections to coincidence. In every other experiment shown in Table IV, in which the residual deflection was zero, at least one more observation was performed after the one recorded. The result was invariable, namely the residual potential was nil at the moment when the negative variation had attained its maximum.

If we assume that, in the injury potential, we are measuring the membrane potential across the boundary of the nerve trunk, the "uninjured" lead being connected to the outside charge, the "injured" lead to the inside charge, then the present result, namely that the injury potential may be just abolished by the negative variation, means no more and no less than a temporary total disappearance of the membrane potential; or, in other words, the temporary total depolarization of the nerve as the result of a tetanic stimulus of short duration.

The absolute depolarization of nerve. The amount of action current "retention" observed in the experiments just described was naturally small owing to the short duration of the stimulus. The question arises, since we know now that a nerve can be temporarily totally depolarized, whether it is possible to depolarize a nerve completely by prolonged stimulation. Putting the question in another way: Can we increase the "retention" until the "retention" is equal to the injury current? This result may best be realized if a sufficiently large number of pairs of stimulating electrodes be provided so as to avoid local fatigue, and if stimulation be applied long enough to exhaust the nerve trunk entirely. Accordingly in the experiments to be described five pairs of electrodes were employed, each pair being used for a period long enough to make up the total duration of stimulation to several minutes. The precautions and the procedure were as described for the case of temporary depolarization. The condition of the nerve plays an even more important rôle than it did there. The results of several experiments of this type are given in Table V. It must be stated that each result given is the highest value obtained in the set of observations referred to. At the end of a long stimulus there was only a small back swing (if any) of the galvanometer, so that in the present case we are dealing with an action current which is entirely "retained." In three cases the residual deflections were practically nil, and in several other cases quite small. We may

TABLE V. Permanent total depolarization produced by stimulation for several minutes at five pairs of electrodes.

Date (1928):										
23. ix.	24. ix.	27. x. (*a*)	27. x. (*b*)	27. x. (*c*)	29. x. (*a*)	29. x. (*b*)	1. xi.	19. xi. (*a*)	19. xi. (*b*)	20. xi.
Injury potential (millivolts):										
3·88	4·35	7·38	5·47	7·74	8·08	8·00	10·07	6·00	5·74	6·74
Deflection due to injury potential (mm.):										
194	235	198	167	283	312	360	487	175	199	193
Duration of stimulation:										
5′ 14″	3′ 0″	3′ 56″	3′ 4″	1′ 13″	2′ 25″	1′ 33″	1′ 52″	4′ 45″	7′ 40″	6′ 30″
Residual deflection (mm.):										
1	22	95	8	85	25	110	160	32	1	66
Percentage depolarization:										
99·5	91	52	95	70	92	70	67	82	99	66

conclude, therefore, that under certain favourable conditions the "retention" of the action current can be made equal to the injury current; or, in other words, an absolute total depolarization of the nerve can be obtained by long continued stimulation, if local fatigue be sufficiently avoided.

The oxidative nature of the injury potential. In view of the structure of, and the nature of the material composing, a nerve fibre, the injury potential can hardly be regarded as due to a surface charge produced by purely physico-chemical causes at a membrane. It is more reasonable to think of it as resulting from a dynamic equilibrium between a constant discharge and a recharge which is effected by the resting activity of the living fibre. The first attempt to identify the source of energy of the injury potential was made as follows. The nerve was placed inside a wax chamber through which passed a constant stream of nitrogen. The gas was purified from oxygen by the method of Kautsky and Thiele(4) as employed by Hill(5), commercial nitrogen from a cylinder being forced through a filter candle into an alkaline solution of sodium hydrosulphite. In nitrogen the injury potential falls gradually and tends to reach a minimum, as shown in Fig. 3. It returns towards, or to, its original value when the nitrogen is replaced by air. The results of thirteen such experiments are given in Table VI.

The second row gives the injury potential at the moment when the nitrogen was turned on; the third row the minimum potential in nitrogen; the fourth the highest potential attained after recovery in air. The percentage drop of injury potential, given in the fifth row, was estimated as follows. As already stated, the injury potential diminishes with time. After the initial rapid change the rate of fall is usually very uniform.

It has been assumed, therefore, that a linear relation obtains between injury potential and time. The probable value of the injury potential

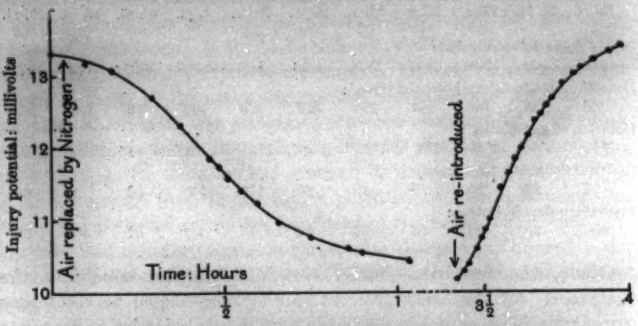

Fig. 3. Fall of injury potential in absence of oxygen: recovery in oxygen.

TABLE VI. Fall of injury potential in absence of oxygen.

Date (1928):	10. ii.	13. ii.	14. ii.	15. ii.	20. x.	24. x.	25. x.	26. x. (a)	26. x. (b)	27. x.	28. x.	29. x.	30. x.
Initial injury potential in air (millivolts):	8·36	14·02	13·40	12·39	13·75	18·58	15·01	27·33	17·47	14·74	16·01	22·00	12·61
Minimum injury potential in nitrogen (millivolts):	3·93	7·45	10·16	6·71	8·82	6·00	6·42	17·06	1·76	5·46	9·09	10·70	5·71
Injury potential after recovery in air (millivolts):	6·77	9·92	13·34	10·76	10·63	10·03	11·51	25·82	6·05	9·91	14·78	19·62	12·41
Percentage drop in injury potential:	49	25	24	40	22	62	51	35	78	55	40	47	54

is then interpolated, for the case of air, for the moment when the minimum potential is reached in nitrogen. From the interpolated value and the observed minimum in nitrogen the percentage drop is obtained. The first four experiments, carried out in February, indicate that with the wax chamber employed a considerable fraction, 24–49 p.c., of the injury potential may be abolished by asphyxia. It was considered, however, that the wax of the chamber might dissolve an appreciable amount of oxygen and give it out again, so contaminating the nitrogen. To avoid this risk further experiments were made in November, in which the wax chamber was replaced by a glass bottle with a thick rubber stopper through which three glass tubes were inserted. One of these was used for the gas exchange and the other two for the necessary electrical connections. The latter two tubes, 10 and 5 cm. long respectively, each

had a side hole made at the inner end and was filled with sea-water gelatin to within 2 or 3 cm. from the free end outside the stopper. The empty outer ends were filled with zinc sulphate solution to receive the galvanometer leads. The nerve was placed on the 10 cm. tube. The injured end was brought in contact with the gelatin inside the tube at the side hole. Connection with an uninjured point was made by a strip of filter paper soaked with sea-water, bridged over from the side hole of the second tube to the first. When the nerve had been mounted the bottle was evacuated with a water pump. It was then filled with hydrogen and evacuated again. This process was repeated several times. The results are shown in the last nine entries of Table VI. The percentage diminution of the injury potential is higher in general than in the experiments of February: the highest value was 78 p.c. The absolute drop of the potential is perhaps more conspicuous, being from 2·5 to 10 millivolts. The average drop is 6·8 millivolts.

The large fall of injury potential in the absence of oxygen, and its recovery when oxygen is readmitted, strongly indicate that oxidation provides the energy required to maintain the potential. The question arises, why is not the injury potential completely abolished by lack of oxygen? In this connection A. V. Hill has recently calculated (6) the depth to which oxygen can penetrate into a sheet of resting muscle exposed on one surface only to a given oxygen mixture. Assuming Krogh's coefficient of oxygen diffusion and a resting metabolism equal to that of frog's muscle, his calculation shows that in nitrogen containing 0·01 p.c. of oxygen the gas should be able to penetrate to a depth of 0·02 mm. Taking the case of a cylindrical tissue ((6), p. 60), and employing Gerard's value for the resting metabolism of nerve, it appears that 20 p.c. of the whole cross-section may be supplied with sufficient oxygen in a gas mixture containing only 0·025 p.c. of that gas. Without the most stringent precautions, therefore, to eliminate the last traces of oxygen, it is easy to understand that there might still be enough present to maintain in sufficient activity a considerable fraction of the whole substance of the nerve.

We have already identified the action potential as a temporary reduction of injury potential, and "retention" as a more permanent reduction, and it has been suggested that the gradual disappearance of the "retention" is due to some kind of recovery process. If, now, the injury potential were ultimately due to an oxidative process we might expect that the gradual disappearance of the "retention" would be much slower in the relative absence of oxygen. It is interesting to note

in this connection that excitability was often lost under anaerobic conditions although the injury potential still persisted. The loss of excitability by asphyxia is a well-known phenomenon in medullated nerve. In Fig. 4 in the upper two curves are shown two different types

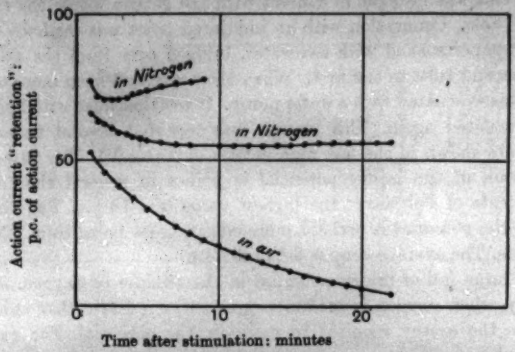

Fig. 4. Disappearance of action current "retention" in presence of oxygen: persistence of "retention" in absence of oxygen.

of time-course of the "retention" process after stimulation in nitrogen. The lower curve, taken in air, is given for the purpose of comparison. Nearly the same maximum deflection was attained in each of the three experiments, the duration of the stimulus being about 20 sec. The initial rapid drop represents merely the back swing on the cessation of the stimulus, the latter not having been long enough to abolish the action current completely. In the case of the experiments in nitrogen the deflection ceases within a few minutes to fall further, then either remains constant or even increases gradually again. In air the curve falls regularly and finally reaches its zero position. There is no doubt therefore that the gradual disappearance of the "retention" involves a restitution process accompanied by oxidation. The injury potential is maintained, and can be restored after stimulation, only in the presence of an adequate supply of oxygen.

In a recent paper Meyerhof describes experiments on the oxygen consumption of Maia's nerve (7). The resting oxygen consumption, and the extra oxygen used as the result of stimulation, are astonishingly high—10 and 20 times, respectively, those of the frog's sciatic nerve at the same temperature. The maximum rate of the extra oxygen consumption due to stimulation reached 40 p.c. of the resting value. Thence it declined slowly until the normal rate was reached somewhere between one-half and three-quarters of an hour,

after a tetanic stimulation lasting 2½ to 10 min. The last result is in good agreement with the conclusion reached above, that the disappearance of "retention" is due to a restitution process involving oxidation.

Discussion. The "negative variation" of the injury current resulting from a tetanic stimulus is produced by the summation of a large number of monophasic action current waves, following one another in rapid succession. Its diminution as the result of prolonged stimulation might conceivably be due to any one of three causes:

(*a*) A prolonged refractory period, allowing fewer impulses to pass per second;

(*b*) a recovery in the injured region, allowing the individual action current waves to become diphasic, so that each second phase would neutralize the first;

(*c*) diminished polarization in the uninjured region, as suggested above.

Of these, (*a*) would afford no explanation of the "retention" effect described by Levin. As shown already, the diminution in the response to a tetanus caused by previous stimulation is exactly equal to the diminution in the injury current also so caused. Thus possibility (*a*) may be dismissed. With regard to (*b*), it seems very unlikely that absence of oxygen would *improve* the condition of the injured region, or that prolonged stimulation at several distant electrodes in succession would produce *complete* recovery from an injury: and one can scarcely imagine that the injured region would then remain completely recovered so long as oxygen is absent. Thus (*b*) also can be dismissed. We are left therefore with (*c*), which we have assumed in the above discussion of the experiments.

The natural way to regard the injury potential is as a potential difference existing across a membrane surrounding the nerve fibre, the "uninjured" electrode being connected to the outside charge, the "injured" electrode to the inside charge. Accepting this view of the matter, we may express our results as follows:

(*a*) The membrane of the nerve fibre may be temporarily depolarized by a short tetanic stimulus;

(*b*) it may be permanently depolarized by a long tetanic stimulus;

(*c*) the building up again of the potential difference involves processes of an oxidative nature and is impossible in the absence of oxygen.

In the injury and action currents, therefore, we are looking at two aspects of one fundamental property of the living nerve cell, its "active membrane potential," the word "active" being used to distinguish it

from other types of membrane potential which may be due to purely physico-chemical causes, differences of concentration, etc.

In crustacean nerve fibres the outside membrane is exceedingly thin, the inside being composed of an apparently homogeneous jelly-like material. A potential difference is maintained across the outside membrane by a "galvanic combustion element" existing either at its surface or in the living material inside (see Straub(3)). The charge involved is continually being dissipated even if the nerve be at rest, just as an accumulator runs down slowly if left standing unused. In a resting condition even a very low oxygen pressure is enough to maintain the potential difference; an insufficient supply of oxygen, however, leads to a fall of potential, as we have found. During the propagation of a stream of impulses, that is, in the rapid discharge of the "active membrane potential," the amount of electricity released may be so great that the capacity of the available battery is not adequate to cope with the sudden demand. The injury potential falls to a low value, or even to zero, and oxidation is not rapid enough to maintain it. Unless oxygen be present to enable the surface layer of the cell to be re-charged, the injury potential and the action potential never rise again to their initial values. In muscle, oxidative recovery restores the capacity for doing mechanical work; in crustacean nerve, apparently, recovery restores to the membrane around the fibre its original electric charge, by which the injury and action currents can be manifested, by which perhaps the impulse is propagated.

Reversible inexcitability not due to stimulation. During the spring, nerves prepared as usual were often found on testing them in the chamber to be completely inexcitable, that is, there was no action current response to stimulation. In August and September, when the phenomenon reappeared, an effort was made to find the probable cause of it, or at any rate to avoid its consequences. It soon became evident that a few minutes' soaking in sea-water immediately after the nerve was prepared could completely prohibit the appearance of this inexcitability, or at least largely mitigate its effect. The mere washing of a nerve in running sea-water for less than a minute often proved to be effective. Some typical cases are shown in Table VII.

TABLE VII. Reversible inexcitability.

Date	Injury potential millivolts	Deflection due to injury potential mm.	Maximum deflection of action current mm.	Percentage "activity"	Remarks
4. ix.	10·99	102	11	11	30 min. after "pulled-out"
	9·13	595	210	35	Soaked in sea-water for 45 min.
	7·86	512	378	73	40 min. interval
5. ix.	7·73	527	5	1	20 min. after "pulled-out"
	5·75	370	.117	31	Soaked in sea-water for 15 min. Interval 40 min.

In both the experiments shown in Table VII the nerves were not at first washed with sea-water but were mounted immediately in a wax chamber after preparation. The maximal deflection obtained on stimulating was only a very small fraction of the injury current, 11 p.c. and 1 p.c. respectively. After the first stimulus they were soaked with sea-water *in situ* for 35 and 15 min. respectively. The recovery of response (see column 5) was remarkable. This type of restoration from an inexcitable state, as the result of washing with Ringer's solution, has recently been reported by Dulière and Horton[8] for the case of a dissected frog's muscle. Gottschalk[9] also has found that nerves rendered inexcitable by asphyxia can be recovered in part either by salt solution or by oxygen, but that complete recovery is attained only when the two agents act together. In the present case the sudden onset of the inexcitable state was not due to exhaustion of the nerve, or to lack of oxygen, for the injury potential before the first stimulation was high. We must suppose, therefore, that inexcitability is somehow produced either by the "shock" of preparation, or by removal from a normal environment, or by some unknown rapid change occurring in the body fluid in contact with air. In crustacean blood there are stated to be so-called "explosive" cells (polynuclear leucocytes) which "explode" spontaneously as soon as the blood is taken from the body. The number of these cells varies greatly from time to time and from one individual to another. The products of the explosion of these cells may conceivably be toxic or narcotic. It is difficult, however, to apply such an explanation to the type of reversible inexcitability found by Dulière and Horton in the case of dissected frog's muscle.

Summary.

1. Levin's experiments on crustacean nerve were repeated and his results confirmed.

2. Injury potential in crustacean nerve diminishes with time, as also does the maximum deflection of a galvanometer recording the sum of the monophasic action currents produced by a tetanic stimulus. The two are connected by an approximately linear relation: both tend to vanish simultaneously.

3. The injury potential is diminished by stimulation (Levin's "retention"). A unit fall of injury potential produced by previous stimulation is accompanied by a diminution in the response to a stimulus which is exactly the same as the deflection due to a unit of potential difference applied externally.

4. By avoiding local fatigue through the use of three pairs of stimulating electrodes a nerve can be temporarily totally depolarized by a tetanus: that is, the maximum deflection due to the action current becomes equal and opposite to the deflection caused by the injury current.

5. Absolute total depolarization can be obtained by long-continued stimulation with five pairs of stimulating electrodes: that is, the action current "retention" may be equal and opposite to the injury current.

6. In the absence of oxygen the injury potential falls: on the

readmission of oxygen it recovers. In the absence of oxygen a considerable part of the action current may be permanently "retained."

7. In crustacean nerve action and injury potentials are two aspects of the same fundamental phenomenon, viz. of an "active membrane potential" existing at the surface of the nerve fibre and maintained by oxidative processes. This potential is discharged by stimulation, or spontaneously at rest in the absence of oxygen. It can be maintained, or restored, only if oxygen be present.

In conclusion I desire sincerely to acknowledge my indebtedness to Prof. A. V. Hill for his suggestion of the present work and for his advice and encouragement throughout it. My thanks are due to Dr E. J. Allen for his permission to work at the Marine Biological Laboratory at Plymouth and for the generous supply of the material used. My gratitude is due also to Mr A. C. Downing for installing the galvanometer employed.

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Note by A. V. Hill.

Experiments made at Plymouth, since the Author left England for Japan, on the heat-production of crab's nerve, have shown that some 98 per cent. of this heat occurs *after*, and only 2 per cent. *during* activity. The recovery heat-production is relatively large, being 20 to 40 times as great as in the frog's sciatic: it occupies, at 13° to 18° C., 25 to 30 minutes after the stimulus (5 to 10 seconds). There is no doubt, therefore, that the gradual restoration to its original value of the injury potential diminished by stimulation, involves considerable energy exchanges and is an active recovery process, involving the consumption of oxygen, as Levin and Furusawa have contended. See also Meyerhof and Schulz(10).

612.45

METHODS FOR TESTING FOR THE PRESENCE OF ADRENAL CORTEX HORMONE.

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(*From the Pathological and Biochemical Laboratories, Cambridge.*)

It is very desirable to have a reliable method for testing for the presence of the hormone presumably secreted by the adrenal cortex.

Most work has been devoted to extending the survival periods of animals previously subjected to double adrenalectomy (*e.g.* Hartman(1)). This method is open to the objection that the survival period is considerably influenced by "heat," the water supply, temperature, etc. To draw reliable conclusions experiments would have to be done on a statistical basis.

(*a*) Recently Swale Vincent and Thompson(2) have reported remarkable effects from adrenalectomy in decerebrated cats. They state that respiration fails within one hour due to the removal of an adrenal hormone—"pneumin"—and that respiration in some cases can be restored by injection of fresh adrenal extracts[1]. If this were true it would offer a convenient method for testing for the presence of adrenal cortex hormone as adrenaline is found to be without effect on the respiration.

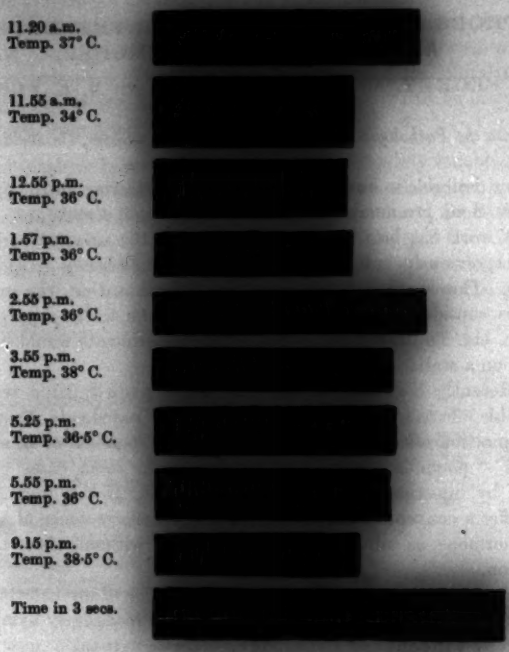
In the course of work involving the decerebration of adrenalectomized cats we noticed that they showed none of the respiratory symptoms described by Vincent and Thompson. Two cats were taken specifically to investigate this point. In one, the adrenals were first removed and then the cat immediately decerebrated, in the other decerebration was performed and then, after a short interval, decapsulation. Subjoined are the protocols.

Cat *A*. 11.40 a.m. to 12.25 p.m. Decapsulation followed by decerebration. Tracings of breathings were taken at 1.15, 2.25, 3.20, 4.15, 5.30 p.m. It was seen breathing well at 6.20 p.m. but found dead at 8.40 p.m. During the experiment the breathing was regular, with an occasional sighing respiration.

Cat *B*. See Fig. 1. 10.28–10.45 a.m. Decerebration.
11.25–11.45 a.m. Decapsulation.

[1] Extracts of any tissue have been found to have the property of stimulating respiration (Drury and Szent Györgyi, unpublished observations).

Hourly records of breathing taken. Alive and breathing well at 9.15 p.m., seen alive at 10.30 p.m. but dead by morning.



At this time good eye reflexes and decerebrate rigidity.
Seen to be alive at 10.30 p.m.

Fig. 1. Records of respiration taken by means of a tambour connected to a side tube on the tracheal canula. For reproduction purposes the tracings have been made clearer by scratching the original marks as carefully as possible with a needle point.

It was clear therefore that these cats did not behave in any way noticeably different from cats retaining their adrenal bodies.

A post-mortem examination did not disclose any accessory adrenal bodies.

(*b*) Among the prominent symptoms in adrenalectomized animals is muscular weakness. Kühl(3) has published experiments utilizing this fact

for testing his adrenal extracts. He used guinea-pigs which were lightly narcotized with urethane and given ether during the operative procedure. The adrenal bodies were removed and later records were made of the effect of short tetani, frequently repeated, on the gastrocnemius muscle.

In an animal retaining its capsules the muscle continued to contract for hours after a fall in height shortly after the beginning of stimulation. The muscle of an adrenalectomized animal after a certain time (about two hours after adrenalectomy) rapidly fatigued and gave no further contractions. Kühl injected extracts of adrenal bodies into the circulation of such an animal and found that contraction recovered to a certain extent for periods up to 40 min. He took certain precautions to destroy adrenaline and states that adrenaline does not cause a return of contraction in a fatigued muscle. Hence he proposed that this rapid muscle fatigue can be used as a useful, objective and rapid test for the presence of adrenal cortex hormone.

We have tried to utilize the method in cats and at first were inclined to support Kühl's views. It is true that if a muscle curve is taken of an adrenalectomized cat at times from six hours to two days after operation fatigue occurs very rapidly.

Our original technique was to remove both adrenals at aseptic operation and then 24–48 hours later decerebrate the animal under ether. A tracing was made from the tibialis anticus stimulated by a faradic current, applied for $\frac{1}{2}$ sec. with $\frac{3}{2}$ sec. interval between stimuli. Sometimes the stimulus was applied to the nerve, at other times to the muscle directly. The muscle was made to work against a rubber band which at the beginning of contraction exerted 120 grm. tension. In all cases we noted a very rapid fatigue of the muscle. If a normal decerebrated cat were utilized the muscle could be made to contract for hours, as in Kühl's experiments.

Fig. 2. Normal decerebrated cat. This tracing shows the effect on the muscle contraction of the tibialis anticus of bleeding 25 c.c. The contractions were restored by the injection of 60 c.c. gum saline solution.

It was suspected however that blood-pressure might play a significant rôle in these results.

Tracings illustrative of the effects of blood-pressure are subjoined.

Fig. 2. This illustrates the effect of bleeding on the muscle curve of a normal decerebrated cat. The contractions disappear, to be re-established when the blood-pressure is restored by the injection of gum saline solution.

Fig. 3 *a* illustrates the effect of blood-pressure on the form and height of the fatigue curve.

Fig. 3 *a*. Normal decerebrated cat. Effect of lowering of blood-pressure by bleeding on fatigue curve of tibialis anticus.

Fig. 3 *b*. Continuation of previous tracing. Shows effect of adrenaline on contractions.

A continuation of this curve (Fig. 3 *b*) shows a phenomenon, the explanation of which may be the specific effect of adrenaline on muscle efficiency or the effects of a previous increase in blood flow. Before the injection of the adrenaline the muscle fatigued rapidly, the blood-pressure being low, but the muscle continued to contract well after the return of B.P. from the acute adrenaline rise to the original low level.

Fig. 4 *a* illustrates the rapid fatigue which occurs in an adrenalectomized animal. The contractions are restored for a short time by raising the B.P. with adrenaline and subsequently by injections of gum saline solution (Fig. 4 *b*). It seems clear that contractions can be restored by merely raising the blood-pressure.

It is stated by Kühl that adrenaline did not restore the muscle contractions in his guinea-pigs. He does not state his dose. We have

Fig. 4 *a*. Cat. Decerebrated 7 hours after adrenalectomy. Shows rapid fatigue of tibialis anticus. Contractions restored by two injections of adrenaline.

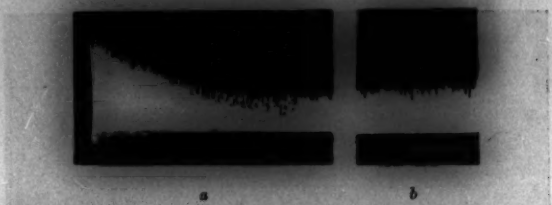
Fig. 4 *b*. Continuation of previous tracing. Shows return of contractions as blood-pressure is raised by injection of gum saline.

evidence that if a sufficient dose of adrenaline be given the contraction of the arterioles becomes so great that the blood supply to the muscle is cut off and the contractions may actually disappear. This fact is partly illustrated in Fig. 3 where the contractions disappear during the height of the rise of blood-pressure.

We have also prepared animals, previously decapsulated, by anæsthetizing with intraperitoneal chloralose and then merely isolating the tendon of the tibialis anticus and suitably clamping the leg. The stimulating electrodes were directly attached to the muscle. Blood-pressure was recorded from the carotid artery. It was found that even with this minimum of interference the blood-pressure rapidly deteriorated.

The work of Gruber (4) indicates that 90 mm. of Hg is the "critical" point, and that the effects of blood-pressure on muscular contraction become increasingly apparent as the former sinks below that level.

It is concluded therefore that Kühl's experiments, unless controlled by a concurrent blood-pressure curve, are equivocal.



a *b*

Fig. 5 *a*. Normal fatigue curve of tibialis in normal decerebrate cat.
Fig. 5 *b*. Tracing after 2 hours of continuous stimulation, compare with Fig. 4 *a*.

This work by no means proves that "adrenal" fatigue is wholly a matter of blood-pressure; that is a question left undecided.

It is admitted that adrenalectomized cats are very susceptible to operative procedures (*e.g.* Hartman(1)), and we feel justified in inferring, from experiments in which the B.P. was measured, that all our adrenalectomized cats had low blood-pressures when the muscle tracing was made.

From the foregoing findings we have concluded that the measurement of muscle fatigue in decapsulated animals by any of the methods currently adopted, does not offer a reliable means for testing for the presence of adrenal cortex hormone in extracts, owing to the extreme susceptibility of adrenalectomized animals to operative procedure, and the consequent difficulty of making a preparation having a blood-pressure of at least 90 mm. Hg.

SUMMARY.

1. A criticism is offered of the statement that decerebrated adrenalectomized cats die in a short time from respiratory failure.

2. The method of measuring the effect on fatigue in the muscles of adrenalectomized animals of extracts of adrenal cortex does not offer a satisfactory test for the content of "hormone" in these extracts.

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ADRENALINE VASODILATATION.

By H. A. DUNLOP.

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It was first shown by Moore and Purinton in 1900 (1) that small doses of adrenal extract may cause a fall of arterial blood-pressure in dogs under the influence of chloroform. Many workers have since demonstrated that a similar effect can be obtained with the active principle. In 1926 MacDonald (2) showed that this effect was obtained notably in animals anæsthetised with ether. The decerebrate animal free from ether or chloroform does not give the depressor reaction.

In 1915 it was observed by Hartman (3) that whereas the splanchnic vessels were usually constricted, the extrasplanchnic vessels were apt to be dilated by adrenaline. Hoskins, Gunning and Berry (4) further showed that the vasodilator effects occurred in the skeletal musculature; these observers found that whereas adrenaline usually caused, in both pressor and depressor dosage, a shrinkage of the intact limb, the skinned limb expanded except with massive doses. At the same time the outflow of blood from the veins of muscles was increased, while the cutaneous venous stream was diminished. That massive dosage may cause expansion of the skinned limb is seen in Fig. 1.

The suggestion does not appear to have been made that the depressor effect of small doses is largely evidence of vasodilatation in muscles, the viscero-cutaneous vasoconstriction being, as a result of anæsthesia, temporarily diminished. This probability was suggested by the observations of Aveling and McDowall (5), that alterations in the electrical resistance of the skin such as can by suitable procedures be obtained in the chloralosed animal could not be obtained under ether anæsthesia. These observations indicated, in view of their explanation of the changes in electrical resistance of the skin, a diminished reactivity of the cutaneous vessels. Blair and Bingham (6) have recently obtained anatomical evidence that the vessels of muscles receive nerve fibres which run independently of those for the skin vessels. It may be noted that Donegan (7) found that whereas the *superficial* and *mesenteric veins*

were markedly contracted by adrenaline in a dilution of 1 in 1,000,000, the *veins of the muscles* were practically unaffected by a solution of the same strength.

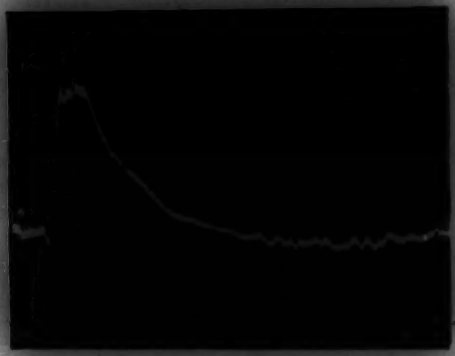


Fig. 1. Cat. Ether. Volume of skinned hind limb and arterial blood-pressure. 0·05 mg. of adrenaline. Time = 10 sec.

The topography of adrenaline vasodilatation.

Experiments have been carried out to determine which blood vessels are chiefly concerned in the vascular dilatation and contraction caused by adrenaline. Etherized cats were used, in which a fall of arterial blood-pressure usually results from intravenous administration of small amounts of adrenaline (about 0·005 mg. for an animal of average size): the fall may or may not be preceded by a slight initial rise. Occasionally as large a dose as 0·05 mg. may cause a fall.

Obstruction of the main abdominal visceral arteries enhances the

Fig. 2. Cat. Ether. Arterial pressure. Hind limbs skinned. At C_1 the aorta was compressed just above the bifurcation, at C_2 the vessel was released. 0·005 mg. of adrenaline before, during and after compression. Time = 10 sec.

depressor response, while clamping the common iliacs diminishes the effect (Fig. 2). Occlusion of the last-named vessels cuts off the cutaneous as well as the muscular blood circulation of the hind limbs. If the hind limbs are skinned the effect of clamping the iliacs in reducing the fall of blood-pressure is more striking than with the limbs intact.

These experiments suggest that the vessels specially dilated by adrenaline, as had been noted by Hoskins, Gunning and Berry, are those of the skeletal muscles. In support of this contention is the shrinkage of the intestine which usually, although not invariably, occurs during the fall (Fig. 3); this shrinkage is considerably less than that obtained with pressor doses. Plethysmographic records show frequently a diminution in the volume of the limbs during the fall of arterial pressure. It has been noted however that the amount of shrinkage recorded is affected by the actual position of the plethysmograph. The more muscle included in the cylinder the less is the shrinkage obtained; the shrinkage is most constantly noted when the volume of the foot alone is taken. When the proximal segment with its mass of muscle is included expansion commonly results. The skinned limb on the other hand expands during the fall of arterial pressure (Fig. 4, *B*); for this result to be obtained, however, care has to be taken to prevent cooling which interferes with and may reverse the reaction.

Fig. 3. Cat. Ether. Intestinal volume and arterial blood-pressure. 0·005 mg. of adrenaline. Time = 10 sec.

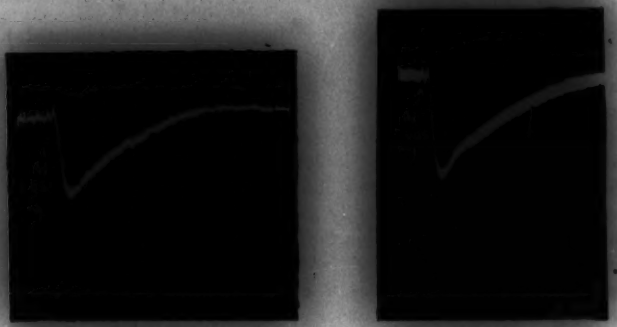
The shrinkage of the intact limb is not the mere result of abstraction of blood to other regions, since the limb may remain shrunken for some time after the arterial tension has passed its lowest value.

The effects of ether on adrenaline action.

Since the experiments outlined above seemed to show that adrenaline causes vasodilatation especially in muscles, the investigation was continued in the hope of obtaining evidence as to how volatile anæsthetics enable small doses of adrenaline to lower the arterial blood-pressure. Two possibilities were considered; anæsthesia either augments the vasodilator action or reduces the vasoconstrictor effect. The *rise* in arterial pressure caused by adrenaline is recognized as resulting from two actions, viz. cardiac and vascular.

The effect of ether on the action of adrenaline on the heart.

In view of the work of Levy(8) which showed that adrenaline was liable to depress the heart in the presence of chloroform, the possibility



A *B*

Fig. 4. Cat. Ether. Arterial blood-pressure, and volume of hind limb (*A*) before and (*B*) after skinning of hind limb. 0·005 mg. of adrenaline. Time = 10 sec.

that ether might have a similar effect was considered. The venous pressure can be taken as an index of cardiac efficiency; the observations

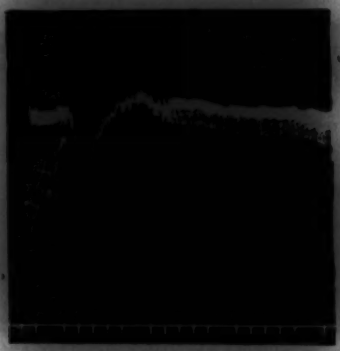


Fig. 5. Cat. Ether. Arterial and venous blood-pressures. 0·005 mg. of adrenaline. Time = 10 sec.

of Eyster and Middleton (9) and also investigations made personally have shown that even slight grades of cardiac impairment cause a rise in venous pressure. During the adrenaline fall of arterial pressure, a fall of venous pressure is the rule (Fig. 5). The venous pressure occasionally shows a slight rise instead of a fall; in this case the rise is delayed till the arterial pressure has well fallen and is not comparable with the rise caused by heart failure. The absence of cardiac impairment is further indicated by the increased force and (occasionally) rate of the heart, as shown by cardiographic tracings (Fig. 6). The output of the ventricles is

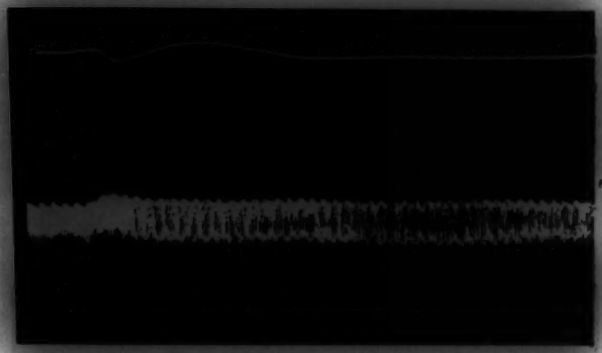


Fig. 6. Cat. Ether. Arterial blood-pressure and heart movements. 0·002 mg. of adrenaline. Time = 10 sec.

slightly increased and their diastolic volume diminished. The absence of heart failure is further indicated by the fact that the pulmonary arterial pressure rises; this change may, however, in part be due to pulmonary vasoconstriction: failure of the left ventricle is excluded as a cause since the cardiographic record shows that the activity of this part of the heart is increased.

The effects of ether on the action of adrenaline on blood vessels.

Theoretically the vasodilator action may be enhanced by ether. There are considerable difficulties in the way of demonstrating such an hypothesis or, alternatively, of disproving it. On the other hand, ether might effect a diminution of the vasoconstrictor action. Dale's work on ergotoxine and adrenaline gave colour to this idea, which was therefore

investigated. Those vessels were studied which adrenaline is known even in small doses to constrict, viz. the splanchnic and cutaneous vessels.

That the *intestinal vessels* are constricted by ether has been shown by Derouaux(10). This I have confirmed. There will thus be less room for further vasoconstriction.

Clinically, ether is known to dilate the *cutaneous vessels*. During the third stage of ether anæsthesia in man the skin is flushed. In decerebrate or chloralosed animals ether causes an increase in limb volume of prolonged duration, which may be preceded by a sharp diminution; effects of ether on the volume of the skinned limb in such animals are much less striking, and usually absent, even when a volume response is readily obtainable by other procedures such as intravenous injection of acetyl choline. It may therefore be concluded that the increase of limb volume caused by ether is due to dilation of skin vessels.

The suggestion that the action of ether upon the vessels of the skin and of the intestine diminishes the constrictor effect of adrenaline may now be considered. For this purpose the effect of a dose of adrenaline on a chloralosed animal was compared with the effect of the same dose on the same animal subsequently given ether. It is found that the shrinkage of the intestine is more marked in the former than in the latter (Fig. 7).

Fig. 7. Cat. Chloralose. Intestinal volume and arterial blood-pressure. Effect of ether on response to 0·04 mg. of adrenaline. At *E* the drum was stopped for 5 minutes and ether administered. At *E off* the drum was again stopped for 5 minutes and the administration of ether discontinued. Time = 10 sec.

The intestinal blood vessels of the decerebrate animal are likewise more strongly contracted by adrenaline than those of the same animal under the influence of ether.

SUMMARY.

It is suggested that ether diminishes the vasoconstriction caused by adrenaline; that ether diminishes the pressor effect had been previously shown by the work of Rous and Wilson(11). The commonly observed differences in reaction of visceral and muscular vessels to adrenaline have been utilized with a view to testing this hypothesis by experiment. Too much stress should not be laid on these differences as being fundamental, but they form a convenient point of attack on a controversial problem. Dale and Richards(12) have given an explanation of the constancy with which adrenaline dilates the vessels of muscle.

It is possible that even with relatively large doses of adrenaline vasodilatation in the muscles takes place, but that it is masked by the preponderant constriction of the visceral and cutaneous blood vessels. However, attempts to prove this in experiments in which a mercury valve was used to control the arterial blood-pressure were inconclusive. Gunning(13) has shown that larger doses than those just mentioned constrict the muscle vessels.

It is further suggested that ether causes a diminution of the cardiac effect of adrenaline, but not conversion of that action from stimulation to depression. It would appear that the lessening by ether of the effect of adrenaline on the heart enables the vasodilator action of this substance to reveal itself in the blood-pressure tracing, but that the principal cause of the fall in arterial tension is a diminution of active vasoconstriction, whereby the dilator action is unmasked.

I wish to thank Prof. McDowall for suggesting the investigation and for much helpful advice. To Messrs Parke Davis I am indebted for supplies of adrenaline.

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THE LETHAL EFFECTS OF ULTRASONIC RADIATION.

By C. H. JOHNSON.

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The rapid destruction of small unicellular organisms and of red blood corpuscles in aqueous media by the agency of ultrasonic radiation was first described by Wood and Loomis(1). Similar observations have since been made by Harvey and Loomis(2) and by Schmitt, Olson and Johnson(3). This effect has invariably been attributed to tearing stresses which the organisms suffer in the standing wave train. The explanation appears quite plausible in the case of the protozoa whose sizes are comparable with the wave-length of the vibrations in water, say one or two millimetres, but less convincing, perhaps, in the case of red blood corpuscles which are relatively minute.

In a recent publication(4) experiments are described wherein oxidations are promoted by irradiating solutions of various substances in water containing dissolved oxygen. Oxidation appears to take place during the *initial stages* of formation of air bubbles expelled from solution by the radiation, that is to say, before the bubbles become large enough to be seen. Experiments were therefore made to find out whether this occurrence was in any degree responsible for the destruction of the organisms aforementioned. The results clearly indicate that the so-called lethal effects of ultrasonic radiation are due mainly, if not entirely, to some peculiar effect connected with the expulsion of dissolved gas from the watery medium, and that the amount of fragmentation caused by mechanical stresses associated with the compressional wave is negligible. A brief account of the experiments will be given. A description of the quartz-crystal oscillator is to be found in previous publications.

Water containing large numbers of the protozoa *Stentor*, *Spirostema*, *Blepharisma* and *Paramœcium* were separately irradiated in a test tube. By connecting the test tube to an oxygen tank external pressure could be applied when desired. Under atmospheric pressure vigorous cavitation of air bubbles was observed, and 30 seconds of radiation sufficed

completely to disintegrate the organisms, as was shown by subsequent microscopic examination. A series of experiments was carried out with applied pressures varying from 10 to 60 lb. per sq. inch. This in no way interfered with the propagation of "sound" through the liquid; but the effervescence of bubbles of visible size was entirely prevented. Up to a certain pressure, total destruction of the organisms took place as before and in the same period of time, but above this critical pressure (which was quite sharp) the protozoa were practically unaffected by 2 minutes of radiation. The critical pressure was found to depend upon various factors, notably the intensity of radiation, but was frequently in the neighbourhood of 60 lb. per sq. inch. (Cf. Schmitt, Johnson and Olson(4).) Carbon dioxide passed for a short time into water containing *Blepharisma* displaced the dissolved air without, apparently, harming the protozoa. No visible cavitation of bubbles occurred on radiating this tube under atmospheric pressure for 2 minutes, and few of the organisms were damaged.

Similar experiments were made with the red blood corpuscles of a rabbit suspended in physiological salt solution. Sharp critical pressures were found for these also. Above the critical pressure the corpuscles collected at the nodes in pretty patterns, and were not destroyed by several minutes' exposure to radiation. Below the critical pressure laking took place in 30 seconds. Displacement of dissolved air by carbon dioxide likewise prevented hæmolysis; but passing pure hydrogen and nitrogen through the liquid for periods as long as 3 hours did not appreciably shorten the time required for hæmolysis to occur. Both hydrogen and nitrogen were cavitated from the solutions irradiated under atmospheric pressure.

Acting upon a suggestion of Dr F. J. W. Roughton of Cambridge University who happened to visit Berkeley while the work was in progress, experiments were carried out with suspensions of blood corpuscles from which the oxygen and carbon dioxide, and dissolved air, were removed by evacuation. Exposure to ultrasonic radiation, under vacuum, produced the "sound patterns" previously mentioned, and after about 5 minutes there was considerable sedimentation. On shaking up the contents of the tube, it was evident that no appreciable amount of hæmolysis had occurred. Air was then admitted (the red colour returned very quickly) and the test-tube shaken for a minute or two in order to saturate the corpuscles with oxygen. Radiating afresh under atmospheric pressure brought about hæmolysis.

Sedimentation takes place fairly rapidly during radiation under

conditions such that the corpuscles are not destroyed, and when the radiation is stopped the clusters remaining in the sound patterns sink to the bottom of the tube. Some very pretty experiments can be made using a tube of 3 mm. diameter in place of a test-tube. Hæmolysis is slow because the radiation density within the liquid is considerably reduced in a narrow tube. Sound patterns quickly appear and then gradually disappear as the hæmolysis sets in; sometimes hæmolysis starts at the top of the tube and spreads downwards, and sometimes the reverse happens. In the former case a curious effect is often observed; hæmolysis will suddenly occur in a narrow region of the tube, the pattern vanishing abruptly as though dissipated by an explosion, perhaps of air bubbles; a few seconds later this is repeated lower down the tube, and so on. When hæmolysis commences at the bottom of the tube, the air bubbles rising through the liquid disturb the sound pattern. Changes in the oscillation frequency of the quartz-crystal between, approximately, 750 and 1000 kilocycles per second, seem not to influence the results; energy intensity is the important consideration.

Conclusions.

It appears that great importance must be attached to the formation of *minute* bubbles of gas within the liquid, caused by the ultrasonic radiation. This can be prevented by application of sufficient external pressure (cf. Schmitt, Johnson and Olson(4)), by replacement of dissolved air with a more soluble gas, or by evacuation. It is suggested that the intense mechanical agitation occasioned at these interfaces by the radiation is primarily responsible for the destructive effects. The production of a powerful oxidizing agent in water containing dissolved oxygen must undoubtedly accelerate the dissolution of micro-organisms, but on reviewing the results as a whole this would seem to be a secondary matter.

A question to be considered in connection with the blood corpuscles is whether the oxygen and carbon dioxide carried by them take part in their destruction; and, again, whether the disintegration of the protozoa is due to the liberation of air bubbles within the cells. Albeit no experiment has been devised to settle the points unequivocally, there is much evidence against these views. In the first place, there is the remarkable parallelism between the phenomena described in this paper and those observed in the experiments on oxidations of organic and inorganic substances in aqueous solution, particularly in regard to the magnitude of the "critical pressures" obtained. Thus, with a given intensity of

radiation and in water saturated with air at 20° C., a critical pressure in the neighbourhood of 65 lb. per sq. inch is necessary to inhibit both the oxidation of iodide ion and the destruction of micro-organisms. Secondly, there are the significant results obtained on saturating with hydrogen and nitrogen, referred to in the text. Lastly, it is doubtful whether any "sound" will be transmitted through the substance of a particle that is free to move about. Energy losses are generally considerable at interfaces and a relatively heavy bombardment by water molecules on one side of the suspended particle will merely drive it towards a node. Hence the sedimentation of blood corpuscles mentioned above, and the rapid settling out of starch granules from aqueous suspensions under the influence of ultrasonic radiation.

It seems likely, therefore, that the lethal action is from without rather than from within; external rather than internal.

SUMMARY.

The destruction of protozoa and of red blood corpuscles by ultrasonic radiation is specifically associated with the expulsion of dissolved gas from the aqueous medium.

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MATERNAL CONTROL OF THE PLACENTAL GLYCOGEN.

By A. ST G. HUGGETT.

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Claude Bernard(1), in 1859, showed that glycogen exists in the placenta of the rabbit. He also demonstrated that it exists in lesser quantity, with a somewhat different distribution in the Ruminants, uterus and trophoblast, and that it appears in the fœtal liver in later uterine life. Pflüger(2) showed, however, that traces were to be found in the fœtal liver before the middle of pregnancy. In 1908 Lochhead and Cramer(3) proved that glycogen is mainly present in the maternal portion, but is found in the fœtal portion of the placenta in minute traces; it was maximal at the 21st–22nd day of pregnancy in the rabbit. After that date it decreased in quantity and percentage in the placenta, while it steadily increased in the fœtal liver. They further showed that the glycogen of the placenta is not increased by feeding with carrots, but that phloridzin injections will lower the glycogen content. They also found an amylolytic ferment in glycerine extracts of the placenta.

The object of this paper is to determine what are the factors which control the deposition of glycogen in the placenta of the rabbit at its maximum on the 21st day, and, further, the agencies concerned in its removal from the placenta in the final period of pregnancy.

The placental deposit of glycogen may be controlled by influences from the mother or fœtus or both. It may be a transition stage for carbohydrate passing through the placenta, or it may be a storehouse for glycogen on behalf of the fœtus. In this case we would expect it to be controlled by the fœtal factors. The maternal stimuli determining the liver and muscle glycogen deposits of the mother may modify the deposition in the placenta. Finally, the control of the placental glycogen may be in no way parallel to other glycogen reserves, but be determined by entirely new factors.

In support of the view that the control may be maternal in origin is the fact that the glycogen is in those placental tissues which arise

from the uterus, that is the decidua basalis, and not in the chorionic portion of the placenta (Maximov(6)).

In this paper some of the possible maternal influences are investigated and comparison is made between the glycogen of the placenta and the glycogen of the muscles and liver.

Methods. The experiments were confined to rabbits, and were performed on the 21st day of pregnancy, at which time it is believed the percentage of glycogen in the whole placenta is maximal.

The glycogen was determined in the whole placenta, no separation of the decidual from the chorionic portions being carried out. The carbohydrate in the fœtal portion being almost negligible, this result served as an index of the maternal placental glycogen.

Glycogen estimations. These were carried out by Pflüger's method. The placenta or liver were dissolved in their own volume of boiling 60 p.c. potash and were heated in a boiling water bath for 3 hours. The hydrolizate was then cooled and diluted with five or more volumes of water. 25 c.c. of this solution forming an exact aliquot part was transferred to a centrifuge tube of 100 c.c. capacity. The precipitation of the glycogen by the two volumes of alcohol and the washing of the precipitate with alkaline alcohol and 66 p.c. alcohol were carried out entirely in the centrifuge tube. It was redissolved in water, the centrifuge tube being heated in a water bath. It was purified by reprecipitation with alcohol and redissolved in water. This solution was then hydrolized by hydrochloric acid in a water bath for 3 hours and the glucose formed was estimated by the Wood-Berry(4) method. The 66 p.c. alcohol used for washing the glycogen contained sodium chloride to prevent loss by solution in the washings.

Simultaneous estimations were performed of the glycogen in the maternal liver, maternal thigh muscles, the whole fœtus, but not the fœtal liver alone. In all cases, except where specifically stated, the pregnant animal was starved for 24 hours before killing.

The normal placental glycogen. The control results on pregnant rabbits killed at the 21st day of pregnancy are given in Table I.

In these results the most obvious facts are the relative constancy of the placental figures compared with the hepatic percentages. Mere starvation for 24 hours in a rabbit (that is removal of all food from the cage) is known to have a variable effect on the liver glycogen (5), especially as there is always a quantity of undigested material of varying composition in the animal's stomach. The second point worthy of note is that the percentage of glycogen in the whole fœtus at that date is rela-

TABLE I. Percentages of glycogen in rabbit's tissues at 21st day of pregnancy after 24 hours' starvation.

	Maternal liver	Whole placenta	Maternal muscle	Whole fœtus
	0·25	3·56	—	0·13
	5·79	3·25	0·23	0·58
	0·09	2·30	0·04	0·15
	1·23	2·40	0·05	0·25
	0·91	3·01	0·02	0·31
	1·91	3·78	0·09	0·22
Average	1·69	3·10	0·086	0·27

tively high. This includes of course the fœtal liver which at the end of the second week of pregnancy is gradually increasing its glycogen stores. It was not separated from the rest of the fœtus for separate estimation on account of the practical limitation of the experiments. The muscle glycogen quantities are in accordance with the results for animals starved for 24 hours (7).

Carbohydrate feeding. Lochhead and Cramer found that the ingestion of carrots had no effect on the quantity of glycogen in the placenta. Their results were confirmed and are shown in Table II.

TABLE II. Placental glycogen at 21st day. Effect of feeding with carrots.

Rabbit	Liver	Placenta	Ratio $\frac{\text{Placenta}}{\text{Liver}}$	Muscle	Fœtus
(i)	6·0	2·48	0·41	0·25	0·25
(ii)	5·88	2·40	0·41	0·05	0·05
(iii)	9·37	3·23	0·34	0·13	0·18

It is evident that there is no parallel between quantities of glycogen in the placenta and maternal liver or maternal muscles. The same applies to the fœtal tissues as a whole. This is well brought out by comparing the percentages of glycogen in the placenta and liver.

$$\frac{\text{Percentage Glycogen in Placenta}}{\text{Percentage Glycogen in Liver}}$$

Normal animal starved for 24 hours $\frac{3{\cdot}10}{1{\cdot}69} = 1{\cdot}84$

Carbohydrate fed rabbit ... ... $\frac{2{\cdot}70}{7{\cdot}08} = 0{\cdot}38$

The higher the ratio the smaller the percentage of glycogen in the liver relative to the placental percentage, and *vice versa*.

Injections of glucose. Attempts were made to increase glycogen by injections of glucose. These were carried out by intravenous and intraperitoneal injections. Again it was found that the glycogen of the placenta varied much less than the glycogen of the liver.

TABLE III. Rabbit's glycogen deposits. Effect of glucose injections.

Dose of glucose grm.	Manner of injection	Liver p.c.	Placenta p.c.	Muscle p.c.	Fœtus p.c.
16	Ear vein slowly	2·88	2·26	0·55	0·78
8	Intraperitoneal	0·50	3·94	0·37	0·36

The glucose used was a 20 p.c. solution in Ringer's fluid.

The action of insulin. The effect of insulin was ascertained. The results are given in Table IV. The dose of insulin varied. In each case it was given subcutaneously in solution containing 2 units per c.c.

TABLE IV. Action of insulin on glycogen stores in pregnant rabbit. Percentages.

Mode of administration		Liver	Placenta	*P/L*	Muscle	Fœtus
Group A.						
2 units per kg. after 24 hours' starvation	(i)	1·89	3·02	1·59	0·66	—
	(ii)	1·74	5·47	3·14	0·0	—
	(iii)	3·77	3·77	1·0	0·0	—
	(iv)	0·18	3·24	18·0	0·026	—
Group B.						
6 units per kg., 3 plus 3 units on the same day	(v)	1·38	1·90	1·37	0·34	0·21
Group C.						
2 units and 3 units per kg. on successive days, 5 units in all with 24–48 hours' starvation	(vi)	0·022	1·80	83·0	0·006	0·009
	(vii)	0·023	1·52	66·8	0·135	0·135

Rabbit (i) was killed after 3 hours. Its blood sugar had fallen from 133 mg. to 26 mg. per 100 c.c., but no convulsions had supervened.

Rabbit (ii) was killed 4 hours after the injection of insulin. It showed no convulsions.

Rabbits (iii) and (iv) were killed 4 hours after insulin and neither showed convulsions. Rabbits (ii), (iii) and (iv) all gave typical sugar depressions following the injection of insulin.

Rabbit (v) was killed 2 hours after the second dose of insulin which itself followed the first dose by 2 hours. It never had convulsions.

Here we first see signs of the placental glycogen being low. Rabbits (vi) and (vii) both had violent convulsions after the second dose, but none after the first dose on the first day. Rabbit (vi) died in convulsions and the estimation was carried out at once. Rabbit (vii) was killed after convulsions had lasted 40 minutes off and on. Here the same rather low placental glycogen was observed as in rabbit (v), but it is nevertheless high compared with the glycogen in the liver which has almost gone and in the muscle of rabbit (vi) which died in convulsions. In rabbit (vi)

incidentally the total fœtal glycogen is reduced. No stress, however, is laid on the significance of these fœtal figures.

It has been stated (Best, Hoet and Marks(8)) that there is an accumulation of glycogen in the liver and muscles when glucose and insulin are given simultaneously. It was therefore of interest to know whether anything similar was observable in the placenta.

TABLE V. Effect of insulin and glucose on rabbit's glycogen stores. Percentages.

Dose	Liver	Placenta	P/L	Muscle	Fœtus
(i) 2 units insulin per kg. and 10 grm. glucose subcutaneous	1·36	3·91	2·8	—	0·12
(ii) 3 units insulin per kg. and 30 grm. glucose intravenous	7·43	3·84	0·52	0·24	0·20

It seems from Table V that in the liver formation of glycogen is stimulated but nothing similar occurs in the placenta.

Adrenaline and glycogen percentage. This is usually considered to have a diminishing effect on the glycogen of the liver (Pollak(9)). The glycogen of the placenta is within the maternal portion and consequently it is of interest to determine the action of adrenaline on the decidual glycogen.

TABLE VI. Effect of adrenaline on glycogen percentages in the pregnant rabbit after 24 hours' starvation.

Dose	Liver	Placenta	P/L	Muscle	Fœtus
(*a*) *Intravenous.*					
(i) 0·01 mg., repeated twice 0·03 mg. in all	2·66	3·06	1·15	0·03	0·05
(ii) 0·10 mg. repeated twice 0·3 mg. in all	0·32	4·45	13·9	0·35	0·49
(*b*) *Subcutaneous.*					
(iii) 0·3 mg. per kg.	2·35	1·84	0·78	0·20	0·04
(iv) 0·3 mg. per kg.	0·39	2·43	6·24	0·06	0·43
(v) 0·9 mg. in two portions of 0·3 and 0·6 mg. at 1 hour interval	0·52	2·68	5·15	0·09	0·29

Rabbits (i) and (ii) killed 2 hours after the last injection, rabbits (iii), (iv) and (v) killed 3½ hours after the last injection.

The effect of adrenaline on the placental glycogen is negative, neither increasing nor decreasing it. The effect on the liver is a diminution of glycogen, but on the placenta it has no such effect. With the maternal muscles there is no such definite change as with the liver.

The thyroid gland. The thyroid gland increases basal metabolism causing an increase of the nitrogenous excretion and of the respiratory

exchange. Feeding thyroid to rabbits has resulted in a decrease of the hepatic glycogen. This was shown by Cramer and Krause(10) in 1913 and has been confirmed frequently since then. The effect on the glycogen of the placenta was tested by giving two rabbits 0·75 grm. of dried thyroid gland (Armour), by the mouth daily for six days. Food was withheld for the last day.

TABLE VII. Effect of 0·75 grm. dried thyroid gland daily for 6 days on the glycogen in the pregnant rabbit.

Liver	Placenta	*P/L*	Muscle	Fœtus	P.c. loss of weight
0·69	1·45	2·1	0·47	0·28	9·5
0·46	2·23	4·85	0·53	0·28	10·1

These two values for placental glycogen are lower than the normal percentages, but the reduction is not marked and is in no way proportionate to the loss in the hepatic glycogen, as shown by the relatively high *P/L* ratios of 2·1 and 4·8 compared with the normal average of 1·84.

Phlorrhizin. Lochhead and Cramer examined the action of phlorrhizin on the placental and fœtal glycogen deposits. They found it caused a diminution in the weight of the glycogen in the placenta and fœtus. This reduction was more marked in the fœtus than in the placenta and most pronounced at the 27th day of pregnancy. The glycogen percentage of the placenta was practically unaffected at the 21st day, but the absolute weight of the placenta and fœtus was decreased, especially at the 27th day. Consequently phlorrhizin caused a diminution of growth of the placenta and of the fœtus, and associated with this there was a diminution in glycogen percentage. This retardation of growth was confined to the fœtal tissues, while the glycogen of the placenta is mainly in the maternal decidual portion. With the thyroid-fed rabbits of Table VII the glycogen percentage diminishes at the 21st day in the placenta. This is associated with a 20 p.c. loss in weight in the mother, but there is no diminution in placental weight as compared with the normal. This is shown in Table IX.

Tetrahydro-β-naphthylamine. Adrenaline caused no disappearance of the decidual glycogen, while thyroid given in repeated doses produced some diminution. It was therefore of interest to investigate the effect of tetrahydro-β-naphthylamine (T.H.N.). The action of this substance is marked by a rise of temperature and by restlessness (Fawcett and Hale White, 1897 (11)). This condition has been described by Cramer (12) as "sympathetic fever" and is marked by a diminution of the liver glycogen.

The T.H.N. was dissolved in warm saline and injected subcutaneously. This was given on the 16th to 21st days inclusive, the animal being killed on the 21st day 2 hours after the injection. The results are shown in Table VIII.

TABLE VIII. Effect of 6 days' administration of tetrahydro-β-naphthylamine on the glycogen percentages at the 21st day of pregnancy.

Dose	Temperature rise	Liver	Placenta	P/L	Muscle	Fœtus
60 mg. per day	1° C.	1·06	0·87	0·82	0·04	1·67
80 mg. per day	1·5° C.	0·043	0·67	15·5	0·066	0·33
80 mg. per day	2·5° C.	0·071	0·91	12·8	0·033	0·48

The most outstanding point is that the placental glycogen is markedly reduced as well as the liver glycogen. This reduction in percentage is not accompanied by a reduction in weight of the placenta or fœtus, and it is therefore a change peculiar to the glycogen itself. It is more pronounced than that of phlorrhizin in the dosage used by Lochhead and Cramer.

It is of interest to correlate the actions of these reagents on the weights of the placenta and fœtus with their effect on the glycogen deposits. These results are tabulated in Table IX. The phlorrhizin is the only reagent that lowers both the fœtal weight and the fœtal glycogen percentage at the 21st day. At this stage it has no material effect on the placenta. The T.H.N. markedly lowers the placental glycogen but has no action on the placental weight or the fœtus, whether whole weight or glycogen percentage. Thyroid feeding like T.H.N. causes a diminution of placental glycogen percentage but the effect is not so marked. The weight is barely affected while the fœtuses are unaffected.

TABLE IX. Weights of placentæ and fœtuses together with their glycogen percentages at the 21st day of pregnancy. Effect of adrenaline, thyroid, T.H.N. and phlorrhizin.

		Placentæ		Fœtus	
Treatment		Weight grm.	Glycogen p.c.	Weight grm.	Glycogen p.c.
1. Normal-average		3·2–5·0	3·10	3·3–5·2	0·27
2. Adrenaline	(i)	4·52	4·45	4·1	0·49
	(ii)	4·48	2·43	6·3	0·43
3. Thyroid	(i)	3·5	1·45	5·1	0·28
	(ii)	4·6	2·23	5·4	0·28
4. T.H.N.	(i)	4·15	0·87	5·2	—
	(ii)	3·4	0·91	4·8	0·48
(23rd day)	(iii)	3·46	0·67	8·4	0·33
5. Phlorrhizin (Lochhead and Cramer)		3·2	3·00	2·58	0·17

N.B. The third T.H.N. rabbit was 23 days pregnant. This accounts for the high fœtal weight. This compares with Lochhead and Cramer's averages of 4·01 grm. placental weight, 1·98 p.c. placental glycogen, 7·20 grm. fœtal weight with 0·28 p.c. fœtal glycogen at the 23rd day.

The next point to note is that the increase in metabolism due to T.H.N. is attributed to sympathetic stimulation. But adrenaline injections did not cause the same diminution in placental glycogen as T.H.N. There was, however, this difference that the adrenaline rabbits had injections only on one day, whereas the T.H.N. rabbits were injected on the five days previous to killing. One of the adrenaline rabbits does show, however, a placental glycogen of 2·43 p.c. which is somewhat low for the placenta. Another small point of interest is the relatively high percentage of glycogen in the fœtus in these adrenaline rabbits. Without stressing the point it is worth noting that while most observers are agreed that adrenaline causes a diminution in the glycogen of the placenta, some workers, notably Drummond and Paton(13) and more recently Kuriyama(14), state that repeated injections of adrenaline cause an increase in hepatic glycogen. The explanation of these discrepancies has not so far been advanced, though it appears to bear some relation to the initial glycogen percentage of the liver and the degree of protein katabolism.

The effects of these reagents were controlled by using a series of normal pregnant rabbits at the 21st day of cyesis. This type of control is open to obvious criticism. It would seem better to control these experiments by using the fœtuses of one horn of the uterus as control against those in the second horn. The objection to this is that the necessary anæsthetic might cause glycogenolysis. However, an attempt was made using amytal as the anæsthetic. The amytal was injected either intraperitoneally or subcutaneously, and 1–3 hours later a small quantity of ether given for induction. This was found to be necessary in all cases of amytal anæsthesia—up to 80 mg. per kg. The abdomen was opened, the fœtuses removed with their placenta from one horn and, the uterus retracting, the horn was returned to the abdomen. There was no post-partum hæmorrhage from the opened horn of the uterus. The abdominal wound was clipped and the drug for the experiment administered. The abdomen was opened 3 hours later and the other fœtuses, placentæ, liver and muscle tissue removed and the glycogen estimated. The results are given in Table X. In these cases rabbits are included which had proceeded beyond the 21st day of pregnancy. This, however, does not vitiate the experiment as an illustration of the use of one horn of the uterus as a control against the remaining horn.

From these results therefore the following facts emerge:

(i) That the liver glycogen percentages are much below normal even in the first rabbit which had had no hormones injected. These low results

TABLE X. Amytal anæsthesia. Laparotomy and unilateral Cæsarean section. Effect of operation, anæsthetic, insulin and adrenaline on the glycogen percentages of the pregnant rabbit.

	Percentages of glycogen							
	Placentæ			Fœtuses			Maternal	
Operation and hormone	Before	After	Difference	Before	After	Difference	Muscle	Liver
1 Anæsthetic and operation only. No hormone. 28th day	0·30	0·10	−0·20	0·32	0·35	+0·03	0·05	0·14
2. Insulin 3 units per kg. 28th day	0·17	0·18	+0·01	—	—	—	—	0·008
3. Insulin 3 units per kg. 21st day	1·83	0·64	−1·19	0·30	0·06	−0·24	0·20	0·54
4. Adrenaline	0·64	0·33	−0·31	—	—	—	—	0·03
5. Normals 28th day Lochhead and Cramer	0·45	—	—	0·63	—	—	—	—

may in the rabbits that had hormone be partly due to the hormone, but the anæsthetic and operative processes have undoubtedly lowered the quantity of glycogen present in the liver before the injection of any hormone.

(ii) This method of control is of no value with the placental glycogen because the initial values are markedly subnormal, showing that the anæsthetic and operative processes are factors removing the glycogen from the placenta.

One reason for variation in these cases is that the placenta sometimes retracts off the uterus and develops a blood clot in between it and the uterine mucosa. In rabbits the placenta is deciduate, so the retraction and separation occur through the uterine mucosa. In those cases where abortion occurred or obvious placental separation had existed at the opening of the second horn, the fœtus and placenta were excluded from the glycogen estimations. Included, however, are all those cases in which there was no obvious and definite separation or subplacental hæmorrhage. Therefore some cases of constricted circulation to the placenta might have been included.

These results on the use of amytal agree with those of Hines, Leese and Baker(15) showing that amytal is an unsuitable anæsthetic for glycogen formation experiments.

Discussion.

It is evident from these results that the glycogen of the placenta varies in a different manner from that of the liver. It is much more stable. Factors which lower or raise the blood sugar have no effect upon it. The onset of insulin convulsions alone does not lower it to any marked degree, but repeated doses of insulin on successive days cause a definite decrease. It is more resistant under insulin than the muscle glycogen.

On the other hand, those measures which might be expected to raise the glycogen stores and which are effective in this way with the liver deposits are quite ineffective with the placental glycogen. Feeding with carbohydrate, injection of glucose alone or with insulin have no such effect in the case of the placental glycogen. It still preserves its constancy.

Thyroid, tetrahydro-β-naphthylamine and to a lesser degree adrenaline are reagents which are marked by increases in metabolism, as shown by the temperature of the body, nitrogen excretion and oxygen consumption. The effect of the first two on the placental glycogen was to cause a definite decrease. With adrenaline this was barely noticeable. With thyroid there was a slight fall to figures of 1·45 and 2·23 p.c., and at the same time there was the marked loss in maternal weight of 20 p.c. In the case of the tetrahydronaphthylamine the fall in the placental glycogen was most pronounced, 0·8 p.c. being the average percentage. In none of these cases was there any evidence of a fall in the fœtal glycogen reserves or of the fœtal weights. Their action has consequently been purely or mainly on the maternal metabolism.

As far therefore as the maternal stimuli are concerned the placental glycogen is very insusceptible to changes and is uninfluenced by ordinary factors of maternal metabolism. But with extreme increase in the endogeneous metabolism or gross demands on the carbohydrate stores, such as repeated insulin injections produce, the glycogen of the placenta slowly disappears, though never entirely under the conditions of the experiment.

Animals dying in insulin convulsions contain glycogen in their placentæ, suggesting that even if it is mobilized in extreme carbohydrate want, it is usually mobilized slowly. The only condition in which it was decreased at all rapidly was where ether and amytal were used as anæsthetics and Cæsarean section performed. In this period of 2 to 3 hours there was an appreciable fall of the placental glycogen. The exact cause of this fall cannot be stated on the evidence available.

SUMMARY.

1. The placental glycogen is remarkably constant in percentage relative to the glycogen of the liver and muscles of the mother.

2. It is unaffected by carbohydrate feeding and by glucose injections, whether alone or with insulin.

3. Depletion of the glycogen of the maternal liver is unaccompanied by a fall or rise in the placental glycogen.

4. Repeated doses of insulin on successive days cause a slight fall.

5. Ether and amytal cause a decrease in placental glycogen.

6. Excessive continued increase in metabolism as in the pyrexia due to tetrahydro-β-naphthylamine or in thyroid feeding over several days causes a decrease in the glycogen percentage, but this is not proportional to the decrease in the glycogen in the liver or muscle of the mother.

7. The glycogen of the maternal portion of the placenta appears to be a reserve for the fœtus which the mother cannot normally draw upon except in extreme cases of disordered metabolism over a prolonged period.

8. These results apply to the rabbit at the 21st day of pregnancy, when the placental glycogen is maximal, but not necessarily to the full term rabbit when it has markedly diminished.

In conclusion, I wish to express my thanks to Dr W. Cramer for suggesting the use of tetrahydro-β-naphthylamine and for giving me some from his stock. I wish further to thank the Beit Memorial Trustees, since it was during the tenure of a Beit Fellowship that this work was initiated.

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THE IDENTITY OF THE RESPONSE OF MUSCLE TO DIRECT AND INDIRECT STIMULATION.

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It is commonly objected to myothermic observations that these have usually involved direct stimulation of the muscle, and that contractions induced by such stimulation are "abnormal." The response to nerve stimulation is held, without much evidence, to be different, and in some way superior. It is true that excessive stimuli directly applied to a muscle produce contracture, delayed lactic acid formation and delayed heat-production (see Meyerhof and Lohmann(1), Furusawa and Hartree(2)), and Hartree and Hill(3) have attempted to explain the delayed heat-production of a muscle contracting anaerobically as due to the over-stimulation of a certain number of its fibres, those lying in immediate contact with the electrodes. In a recent paper Fischer(4) claims to have established the existence of such a difference between direct and indirect stimulation. According to Fischer single shocks, or short tetani, directly applied to the muscle, evoke the same tension as, but somewhat more heat than, similar stimuli applied to the nerve of a frog's sartorius preparation. The difference claimed is not large, averaging in one table only 6·5 p.c. and being rather variable. The accuracy, however, of the myothermic method is relatively so high that the existence of such an error (ranging from 0 to 15 p.c.) would be a definite objection to direct stimulation. The following observations therefore were made in order to test the accuracy of Fischer's conclusions, and to determine whether, in fact, the delayed anaerobic heat can be attributed to over-stimulation of a certain number of the fibres.

The experiments proved unexpectedly easy and decisive. Hartree and Hill(3) raised against indirect stimulation of a sartorius preparation the objection that no security exists that any difference which might be observed to occur between direct and indirect excitation could not be attributed to the injury (or asphyxia, if under anaerobic conditions) of a certain proportion of the fibres of the nerve. The present experiments, however, have shown, with surprising sharpness, that no

such differences do in fact occur, so that the objection has no weight. A sartorius muscle, prepared sufficiently cautiously with its nerve and excited with maximal (but not supermaximal) stimuli, behaves in all respects precisely in the same way whether the stimuli be applied directly to the muscle, or indirectly through the nerve.

A suggestion by Prof. Adrian was adopted, that the nerve should be prepared with a small amount of adherent tissue, in order to avoid mechanical strain upon it. The contractions employed were isometric, both for the sake of greater accuracy in the heat measurements and to avoid displacement of the nerve. The muscle (a single sartorius) lay upon a thermopile with its usual electrodes for direct stimulation, and the nerve was placed on a pair of fine electrodes, 2 and 3 mm. respectively from the muscle.

Table I shows the initial heat H and the maximum tension T for

TABLE I. Initial heat (H) and maximal tension (T) in arbitrary units, in single maximal twitches evoked by direct (M) or indirect (N) stimulation.

Date	Temp. °C.		N	M	N	M	Mean N	Mean M
20. ii. 29	0	H	372	372	372	—	372	372
		T	142	142	143	—	142½	142
25. ii. 29	0	H	326	324	323	—	324½	324
		T	111	110	110	—	110½	110
„ later	0	H	322	320	319	—	320½	320
		T	107	106	107	—	107	106
26. ii. 29	0	H	—	446	442	446	442	446
		T	—	139	136½	136	136½	137½
7. iii. 29	18	H	247	255	253	—	250	255
		T	92	91½	90	—	91	91½
11. iii. 29	18	H	—	260	256½	257½	256½	259
		T	—	85	83½	83½	83½	84

several cases of single twitch. Each number entered in the table is the mean of two or three readings of the same kind (nerve or muscle stimulation) and in every case the first kind of stimulus was repeated, after the second kind, so that, by averaging, any progressive change can be eliminated. Preliminary trial ensured that the shocks employed were, in each case, and for both kinds of stimulation, just and only just maximal.

In other experiments not quoted the correspondence was as good, and there were no cases showing any marked divergence in the results obtained from the two kinds of stimulation. Similar experiments were

made for the case of tetanic stimulation. For example, for 0·2 sec. tetanus at 0° C.:

				Mean	
	M	*N*	*M*	*N*	*M*
H	566	559	560	559	563
T	135	134	132½	134	134

and for various times of tetanus *t*, in seconds, at 0° C. in two different experiments:

	Indirect				
t	0	0·1	0·5	1	1·5
H	407	532	810	1034	1250
T	129	149	164	167	167

	Direct				
t	0	0·1	0·5	1	1·5
H	406	528	820	1060	1280
T	130	150	166	167	167

	Direct			
t	0·1	0·5	1	1·5
H	471	725	968	1140
T	129	143	147	147

	Indirect			
t	0·1	0·5	1	1·5
H	475	728	940	1125
T	130	143	144	145

In these experiments mean values are shown, with "reverse" in respect both of time and of kind of stimulus.

It should be noted that the above heat readings have not been corrected for the heat produced by the stimulating current. In the case of a single twitch this is negligible. For a tetanus of 1 second it was estimated as 14 units (about 1½ p.c.), by "stimulating" the muscle when dead: allowing for this makes the agreement even better.

It is clear that the method of stimulation, if maximal but not too strong, has no detectable effect either on the tension or on the heat-production.

To see if there was any difference in the delayed heat under anaerobic conditions due to the method of stimulation, various experiments were carried out. Ordinary cylinder nitrogen was used, in which asphyxiation of the nerve occurs, if at all, only after a very long time. A certain small amount of recovery heat, due to traces of oxygen, may have appeared in these cases, but since comparison was made directly between the galvanometer deflection-time curves for direct and indirect stimulation, the same error, if any, would occur in each, and would have no effect. To exhibit the results all the curves of galvanometer deflection have been reduced to a maximum of 500 mm.: this was approximately the reading in each case, a resistance being introduced into the galvanometer circuit when the heat-production was larger. In the experiments readings were taken every 5 seconds up to 2 minutes, and then at longer intervals: for the comparison, however, it is sufficient to quote

only a few readings. One typical experiment gave the following curves:

Time: mins.	...	0	¼	½	1	1½	2	2½	3	3½	4	5	6	7	8	9	10
0·5 sec. tetanus	*N*	0	354	202	81½	46	32½	26	22	19	16	11	7	5	3	2	1
„ „	*M*	0	355	201	82	45	31½	26	22½	19½	16½	11½	8	5½	3½	2½	1
8 shocks in 1 sec.	*N*	0	345	185	67½	34	23	19	15½	14	12	9	6	4	2½	1½	1
„ „	*M*	0	346	186	67	34½	24	18½	16	14½	13	10	7½	5½	3½	2	1

This experiment showed, as usual (cf. (3) and (5)), a much greater delayed heat in the case of a tetanus than in that of a set of twitches, but no significant difference between direct and indirect stimulation.

In other experiments of this kind it will be sufficient to show the difference between the curves *M* or *N* and their mean, to the nearest mm. (maximum 500). All these experiments were at 18° C.

Time: mins.	...	¼	½	1	1½	2	2½	3	3½	4	5	6	7	8	9	10
3 shocks		0	2	1	1	1	0	0	0	0	1	0	1	0	0	0
0·05 sec. tetanus		6	2	0	1	2	1	0	0	0	0	0	0	0	0	0
5 shocks		4	0	0	0	0	1	1	0	0	0	0	1	1	0	0
0·2 sec. tetanus		1	1	0	0	1	1	1	2	1	0	0	1	1	0	0
7 shocks		2	2	3	1	0	1	1	1	0	0	0	1	0	0	0

This fully confirms the statement that there is no observable difference, in the response to a just maximal stimulus, between indirect or direct stimulation.

It is not easy to see why Fischer reached the contrary conclusion unless his induction shocks were slightly too strong, and either liberated heat physically or caused an abnormal response to excessive stimuli. The present writer may perhaps be allowed to emphasize that he started with no prejudice in favour of the results recorded here, which in fact contradict the suggestion he made with Hill(3) to explain the delayed anaerobic heat. That heat, whatever be its cause, is not due to excessive direct stimulation of some of the fibres of the muscle, since it occurs equally when stimulation is applied through the nerve. It is satisfactory, however, that direct stimulation with carefully adjusted maximal shocks is justified, since this has been employed in almost all recorded myothermic observations.

Summary.

The heat-production, initial and delayed, and the tension developed, in the isometric contraction of a frog's sartorius, are precisely the same whether the muscle be excited directly, or through its nerve, provided that the stimuli are carefully set to be just maximal. The suggestion,

therefore, by Hartree and Hill that the delayed anaerobic heat after a tetanus is due to the excessive stimulation of a fraction of the fibres is incorrect, since this heat appears equally when the muscle is excited indirectly.

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THE SIGNIFICANCE OF THE OCCURRENCE OF ŒSTRIN IN MALE URINE.

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I. Introduction.

The work recorded in this paper was concerned with three related problems: (*a*) the reported presence of an œstrus-producing substance in male urine, and its identity with that obtained from female sources, (*b*) the significance of the occurrence in the male of what has been regarded as a female sex hormone, and (*c*) the nature of the process by which œstrin reaches the urine.

As regards the occurrence of an œstrus-producing substance in the male, it has been reported as present in the testis (1, 3), the blood (4, 6) and the urine (7, 8, 10). In most cases, however, vaginal cornification appears to have been the only criterion of œstrus used, and since this reaction is now known not to be entirely specific for œstrus (2) it seemed desirable to test extracts from male sources by at least one other criterion of œstrus. Investigation showed that the substance present in male urine was almost certainly œstrin, and it was then endeavoured to ascertain whether it had any essential connection with the testis, or whether its occurrence in the male was merely accidental.

Finally, the renal secretion of œstrin was studied by means of the isolated heart-lung-kidney preparation.

II. The presence of œstrin in male urine.

Samples of urine were collected from individual workers in the laboratory over a period of about 8 hours. Each sample was preserved by the addition of a little toluene during the collection. As soon as possible after collection the specimens were evaporated to a small bulk under reduced pressure and the concentrated residue dried with anhydrous

Na_2SO_4. This dried material was then thoroughly extracted with ether for several hours in a Soxhlet apparatus. The ether extract, after washing 4–6 times in a funnel with water, was evaporated to dryness, the last traces of solvent being removed *in vacuo*. The thorough washing of the ether extract with water has been found to be of considerable importance, since the extracts proved to be highly toxic to mice when this precaution was not taken.

The residue, after drying, was again treated with about 5–10 c.c. ether and filtered from small quantities of amorphous pigments that were not readily soluble in ether. The filtrate was then again evaporated to dryness, weighed and emulsified in 1–2 c.c. of 0·5 p.c. Na_2CO_3. The addition of a few drops of olive oil was found to result in smoother and more stable emulsions. This emulsion was then injected subcutaneously into one or two ovariectomized mice and the vaginal smears recorded every 12 hours. The results are shown in Table I.

TABLE I.

No. of extract	Source	Vol. urine c.c.	Weight total ether soluble material g.	Weight injected into each mouse g.	Mice used (O.M.)	Result
U.M. 5	A	1150	0·291	0·14	97	Cornified vaginal smear
					98	„ „
„ 6	B	800	—	—	113	Negative
					114	Cornified vaginal smear
„ 7	C	795	0·185	0·09	50	Negative
„ 9	C	630	0·199	0·09	53	Cornified vaginal smear
					54	„ „
„ 10	A	730	0·115	0·06	55	„ „
					57	„ „
„ 11	D	855	0·214	0·10	82	„ „
					84	„ „
„ 12	A	975	0·199	0·09	85	„ „
					86	„ „

These results confirm those of Loewe(10) and of Laqueur(8) and their co-workers who showed that substances producing cornification of the vagina of ovariectomized animals can be obtained from the urine of normal healthy human males. It was felt, however, that it would be unjustifiable to assume the identity with œstrin of the substance responsible unless criteria of œstrus other than cornification of the vagina were also satisfied. Examination of several of the test mice that gave positive vaginal reactions showed the uterus to be in the typical anœstrous condition. However, these animals were all killed towards the end of the induced cornification and any effect on the uterus may have passed

unobserved. In addition, from observations carried out on mice injected with placental œstrin, it seemed possible that amounts of the hormone sufficient to produce cornification of the vagina might be insufficient to produce growth of the uterus. Accordingly it was decided to work up a large amount of male urine and inject into a number of mice quantities of the extract which would undoubtedly cause growth of the uterus if the substance concerned was œstrin.

Forty litres of urine from nine different workers in the laboratory were collected under toluene and extracted four times with ether as soon as possible after collection. The ether was evaporated, and the aqueous residue heated for 10 minutes with 100 c.c. of 5 p.c. aqueous KOH. This partially saponified mixture was cooled, diluted with water and extracted with ether four times. The ether extract after being washed four times with water to remove excess of alkali and soap, was evaporated to dryness *in vacuo* and weighed. In this way 0·1456 grm. of a brown oily material was obtained. A portion of this was emulsified in 0·5 p.c. Na_2CO_3 with the addition of a few drops of olive oil and injected into two ovariectomized mice. Each mouse received about 24 mg. of the extract, which was given in six separate injections over 36 hours, in order to prolong the action. Vaginal smears were recorded every 4 or 5 hours. Both animals were killed 5 hours after the first signs of cornification in the vagina. In each case the uterus was in the typical swollen condition characteristic of œstrus.

There can be little reason to doubt that the substance occurring in male urine responsible for these phenomena is indeed identical in its physiological action with œstrin prepared from ovaries or placenta.

The problem now arose as to the source of this œstrin in the male. Two possibilities presented themselves. (*a*) That it was produced in the body. (*b*) That it was present in the food and was absorbed through the alimentary tract. The first hypothesis receives some support from the reports of the presence of œstrus-producing substances in testis (3). On the other hand, the fact that œstrus-producing substances can be obtained from various plant sources (5, 11) and that œstrin may have a slight activity when administered by the mouth (9) lends support to the latter alternative.

An attempt was made to investigate this problem by collecting and testing the urine of male rabbits before and after castration. Five extracts were made from quantities of urine from normal male rabbits, varying from 1500 to 3100 c.c., in the manner described in the last experiment. Each extract was injected into two ovariectomized mice.

It was found, however, that these extracts were frequently highly toxic and, in addition, only one of the five extracts made proved to have any œstrus-producing activity at all. Since it seemed to be impossible to demonstrate the presence of œstrin in the urine of the normal male rabbit with any regularity, this experiment was abandoned.

III. Renal excretion of œstrin.

McClendon, Burr and Conklin(12) state that œstrin may be detected in the urine some few hours after subcutaneous injection, but little information appears to be available with regard to the mechanism whereby it gets into the urine.

It was therefore decided to study the excretion of œstrin on the isolated kidney.

For this purpose the isolated heart-lung-kidney preparation was used. The operative technique was essentially the same as described by Starling and Verney(14). The kidneys were perfused at arterial blood-pressures between 90–120 mm. Hg and the renal blood flow varied between 80–120 c.c. after the urine secretion started. Blood temperature was kept between 36·0–37·5° C. Usually 500–1000 c.c. of blood were in circulation. When the urine flow had definitely started after the insertion of kidney into the heart-lung-circuit, from 1–2 c.c. of urine were rejected in order to avoid contamination with any urine previously secreted and remaining in the renal pelvis and tubules. A 10 c.c. sample of urine was then collected (usually during the next 15–30 minutes), after which either 100 rat units or 200 mouse units of œstrin were added to the circulating blood. A further one or two 10 c.c. samples of urine were collected during the next 20 minutes. Blood samples, each of 10 c.c., were also taken before and after the addition of the œstrin. These were extracted and tested for their œstrin content. The results are summarized in Table II.

Table II. Renal secretion of œstrin in heart-lung-kidney preparation.

No. of exp.	Blood in circulation c.c.	Amount of œstrin added	Amount of urine sample			Rate of secretion c.c. per 10 mins.			Urine test		
			1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
1	600	100 r.u.	7·5	9·8	—	3·7	7·5	—	Neg.	Active in 5 c.c.	—
2	550	120 r.u.	14·0	12·0	12·0	7·8	8·6	15·0	„	Active in 6 c.c.	Neg.
3	1000	200 r.u.	10·0	10·0	—	5·0	10·0	—	„	„	—
4	1640	200 m.u.	10·5	20·0	20·0	5·2	—	40·0	„	Neg.	Neg.
5	450	200 m.u.	20·0	20·0	—	8·0	5·0	—	„	„	—

The following conclusions may be drawn from these results:

(*a*) Small amounts of œstrin may be found in the urine shortly after a large dose has been added to the circulation. Only about 1 p.c. was, however, recovered from the urine. The isolated kidney, therefore, does not actively secrete or concentrate the hormone. Its occurrence in the urine is probably the result of diffusion through either the glomerular or tubular epithelium.

(*b*) The hormone disappears from the blood of the heart-lung preparation very rapidly. It was detected in none of the samples of blood that were taken.

To throw further light on the fate of œstrin in the preparation, two further sets of experiments were performed: (*a*) addition of œstrin to standing blood and subsequent extraction, (*b*) addition of œstrin to a heart-lung preparation and extraction of the component parts, heart, lung and blood. The results showed:

(*a*) That œstrin is not appreciably destroyed by incubation in standing blood for 3 hours either at room temperature or at 37° C.

(*b*) Only a small amount of œstrin (? 3 units and ? 2 units respectively) is retained by the heart and lung tissue, and a 25 c.c. blood sample gave negative results 25 minutes after the addition of 200 M.U. to the 425 c.c. of blood in circulation.

Œstrin added to a heart-lung-kidney preparation is thus not excreted, or adsorbed by the heart and lung, in appreciable quantities, and since it is not destroyed in standing blood, it is probable that the very rapid destruction observed is due to oxidation as the blood circulates through the lungs.

Since such a small percentage of the œstrin added to the blood of such a preparation is eliminated by the kidneys the presence of large amounts in the urine of pregnant women suggests that very large quantities must be produced at this time.

We wish to thank Prof. E. C. Dodds who kindly supplied us with much of the œstrin used in the excretion experiments.

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STUDIES ON OVULATION. I. The relation of the anterior pituitary body to ovulation in the rabbit.

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I. Introduction.

The relation between the anterior pituitary and the ovary. While working on the growth promoting factor of the anterior pituitary body Evans and co-workers (1, 2) found that sodium hydroxide extracts of ox anterior lobe brought about complete cessation of the œstrous cycle in the rat. This effect was due to the luteinization of the epithelium of all the larger follicles without the intermediate act of ovulation. As a result of this the ovaries of treated animals came to consist almost exclusively of vast numbers of atretic corpora lutea. The presence of this luteal tissue was apparently responsible for the inhibition of œstrus. Subsequently Smith and Engle (6) and Zondek and Aschelm (8) demonstrated an entirely opposite effect of anterior pituitary substance. These authors were able to produce the normal maturation and ovulation of large numbers of follicles in both the immature and adult mouse by daily intramuscular implantations. In the former the precocious occurrence of œstrous symptoms coincided with the premature ovulation.

Both these types of reaction have been obtained by other workers, but it is uncertain as yet whether one or more anterior pituitary substances are concerned in the regulation of the ovarian cycle. It is quite evident, however, that ovarian periodicity is to some extent dependent upon the anterior pituitary body. The only observations on the effect of hypophysectomy on the œstrous cycle appear to be those of Smith (5) who found anœstrus in the rat after the operation. Most of the work on this problem has been done on the rat and mouse, but peculiarities in the reproductive processes of the rabbit seem to make this animal very suitable for such work.

Ovulation in the rabbit. It was long ago shown by Heape(4) that ovulation in the rabbit only takes place after copulation, and this observation has been frequently confirmed. In the absence of the male œstrus persists through the entire breeding season and the only cycle found is the alternation of œstrus and anœstrus. The exact mechanism whereby the act of copulation causes ovulation to occur within 10 to 14 hours (2) has never been adequately worked out, but in view of the work mentioned above it would seem that the pituitary body must be concerned, and that the effect of copulation is not exerted directly upon the ovary by nervous or other means. It is still necessary, however, to suppose that the immediate effect of copulation is nervous (artificial insemination does not result in ovulation (3)) and the action is presumably exerted upon the anterior pituitary body. On such a view ovulation in the rabbit depends upon two consecutive events, the first of which is nervous and the second endocrine. The experiments which are recorded here deal with the effect on ovulation of removing the anterior pituitary body at different intervals after copulation.

II. Method and materials.

Animals. Mature rabbits were bought direct from dealers and isolated for at least a month before any attempt was made to use them. By this means the difficulty of their previous history being unknown was overcome and a good supply of animals in œstrus obtained. Nevertheless, a few animals which later proved to be immature or pseudopregnant were used. These, however, were always detectable from examination of the ovaries, uterus and mammary glands and are omitted from the experimental records.

Operative technique. The fact that the final maturation of the follicle is comparatively rapid and that the time of ovulation can be calculated to within an hour or two suggested that the problem could be treated by means of acute experiments. The method finally adopted to remove the pituitary body was as follows. The animal was placed under deep chloroform anæsthesia, the carotid arteries and external jugular veins ligatured and a tracheal cannula inserted. The skin of the head was then removed in the region of the thyroid cartilage around to the base of the ear on both sides and the anterior part of the head removed with a hack-saw; the cut being made immediately behind the temporo-mandibular articulation to a point just posterior to the eyes. Hæmorrhage was arrested during the operation by compression on the vertebral arteries and later by the application of hot saline swabs. Such a cut transects

the brain immediately posterior to the optic chiasma and anterior to the pituitary fossa, removing all of the frontal lobes and part of the temporal and parietal regions. After practice this operation can be done with little or no hæmorrhage and the level at which the brain is transected determined quite accurately. Such animals will live for upwards of 24 hours provided that an anæsthetic dose of urethane is given every 4 hours to inhibit the reflex movements characteristic of the decerebrate rabbit. Temperature must also be regulated to within 37·0° to 39·0° C. The ultimate cause of death in undisturbed preparations was either circulatory failure resulting from sepsis or else secondary hæmorrhage. This technique has, for our purpose, several distinct advantages over the usual trephine method employed in brain operations. In the first place owing to the small size of the rabbit's skull it is very difficult to ensure both a rapid and complete removal of the pituitary body when approached in the usual manner; the partial decerebration method was only used because it afforded an easy route to the pituitary body and made the severity of operation practically the same in the control and hypophysectomized animals. To effect hypophysectomy the anterior wall of the pituitary fossa was broken down and the pituitary body shelled out. Usually it was possible to remove all of the gland in a single piece.

In earlier experiments the ovaries were exposed before the calculated time of ovulation in order that continuous observation could be maintained, and in a few animals the ovaries were removed at different times to provide histological material at two stages from the same animal.

The ovaries, together with portions of the uterus and vagina, were fixed in Bouin's fluid and the sections stained with hæmotoxylin and eosin. In the earlier experiments the ovaries were fixed and imbedded whole, but later, to avoid the labour of cutting complete serial sections, only the essential portions of ovaries were imbedded. Complete serial sections were cut through the selected tissue from critical experiments.

III. Experimental results.

Unoperated animals. A number of initial observations were made on normal animals anæsthetized about the time of ovulation. The ovulation time found in these was essentially the same as that recorded by Walton and Hammond (7) (see Table I).

Although copulation will sometimes take place during pregnancy or pseudopregnancy we have failed, in common with other workers, to find ovulation at this time.

TABLE I. Ovulation after copulation in the œstrous rabbit.

No. of animal	Time of killing after copulation hr. min.	Time of ovulation	State of ovary at death
APO 13	10 30	—	Large follicles. No ovulation
APO 14	12 35	?	Ovulated
APO 36	25 27	11 : 00 to 11 : 45	Young corpora lutea

Operated animals with anterior pituitary intact. Seven animals are available which survived partial decerebration for 12 or more hours after copulation. The results are summarized in Table II.

TABLE II. Ovulation with anterior pituitary intact.

No. of animal	Time of decerebration after copulation hr. min.	Time of ovulation	Duration of experiment hr. min.	State of ovaries at death
APO 22	0 28	—	12 05	Follicles hæmorrhagic
APO 29	0 30	14 : 05–17 : 15	21 00	Young corpora lutea
APO 52	1 42	11 : 00–11 : 30	11 50	" "
APO 7	2 15	12 : 15 (or earlier)	12 15	" "
APO 11	2 40	10 : 35–12 : 10	15 10	" "
APO 12	3 05	12 : 20–12 : 40	14 50	" "
APO 17	6 45	10 : 10–11 : 25	16 35	" "

From these results the following conclusions may be drawn:

(*a*) No operative effect can be observed on the occurrence or the time of ovulation.

(*b*) The involution of the ruptured follicle and the formation of the young corpus luteum is normal.

(*c*) Histological observations failed to reveal any abnormality in the final maturation and rupture of the follicle (see Plate I, figs. *a*, *b*, and *d*). The secretion of secondary liquor folliculi, the separation of the discus proligerus and the hæmorrhage in the follicle wall just previous to its rupture, all proceeded normally.

Operated animals with the anterior pituitary removed. Ten animals are available which lived beyond the normal ovulation time with the anterior pituitary removed. The results are summarized in Table III.

The following conclusions may be drawn from these experiments:

(*a*) Hypophysectomy within the first hour after copulation inhibits rupture of the follicle for at least 16 hours beyond the normal ovulation time and probably permanently.

(*b*) Hypophysectomy later than the first hour after copulation does not appear to influence the occurrence or time of ovulation.

TABLE III. Ovulation with anterior pituitary removed.

No. of animal	Time of decerebration after copulation hr. min.	Duration of experiment hr. min.	State of ovaries at death
APO 46	0 13	16 19	No ovulation
APO 20	0 16	13 10	,,
APO 42	0 18	22 00	,,
APO 40	0 19	16 00	,,
APO 44	0 25	16 00	,,
APO 41	0 32	28 00	,,
APO 30	1 30	16 00	Ovulated
APO 16	4 35	16 50	,, (at 12 hr. 05 min.)
APO 27	6 30	15 05	,,
APO 26	6 50	18 50	,,

(*c*) Histological examination suggests that where ovulation occurred the corpora lutea (presumably owing to the absence of the anterior pituitary body) did not show normal development. We hope to investigate this aspect of the problem in more detail later.

(*d*) Histological examination of those follicles which did not ovulate showed that atretic changes occur after the time when ovulation should have taken place (see Plate I, fig. *c*). These changes consist of general shrinkage, resulting in crumpling of the follicular wall, and in disintegration of the membrana granulosa and of the corona radiata, together with vacuolar degeneration in the ovum. The changes in the follicular epithelium are of the same nature as those which precede normal ovulation, but they are carried much further. We have satisfied ourselves that the extensive degeneration observed in these experiments is neither of post-mortem origin nor due to inferior histological technique. The ultimate fate of the degenerating follicles has not been observed owing to the limitations of our operative technique.

IV. DISCUSSION.

It will be noticed that although both the anterior and posterior lobes of the pituitary body were removed, it has been assumed that the anterior lobe was responsible for the differences observed between the control and experimental animals. This could be objected to on the grounds that removal of the posterior lobe might cause circulatory changes sufficient to inhibit ovulation. No such changes, however, could be detected by observation of the heart rate and general arterial blood-pressure; nor was there any appreciable difference in the appearance of the ovaries and viscera of the control and hypophysectomized animals.

It is too early to discuss the bearing of these experiments on the

problem of whether one or more substances are secreted by the anterior pituitary body for the regulation of the ovarian cycle, but it may be pointed out that copulation must immediately cause either an increased secretion of the substance concerned in the initial maturation of the follicle or the secretion of another substance concerned solely with the final stages of maturation and the luteinization of the follicular epithelium.

V. Summary.

1. Hypophysectomy in the rabbit within 1 hour after copulation inhibits ovulation, which would normally take place 10 to 12 hours later.

2. Hypophysectomy later than 1 hour after copulation has no such inhibitory effect, although the subsequent development of the corpora lutea appears to be abnormal.

3. Mature follicles which fail to ovulate owing to early hypophysectomy begin to undergo atresia after the normal ovulation time.

4. In view of previous work on the relation between the pituitary body and the ovary these effects may be attributed to the removal of the anterior lobe.

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Guide letters: *C.R.* Corona radiata; *D.P.* Discus proligerus; *G.E.* Germinal epithelium; *H.* Hæmorrhage; *M.G.* Membrana granulosa; *O.* Ovum; *T.E.* Theca externa; *T.I.* Theca interna.

Fig. *a*. Mature follicle (APO 28) at time of copulation, showing relation to surface of ovary.

Fig. *b*. Follicle about to rupture (APO 22) 12 hours after copulation, showing hæmorrhage at point where rupture will take place. Pituitary body intact.

Fig. *c*. Follicle 22 hours after copulation (APO 42). Ovulation inhibited by removal of pituitary body. Note degenerative changes.

Fig. *d*. Young corpus luteum 16½ hours after copulation (APO 17). Pituitary body intact.

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THE ASSAY OF ŒSTRIN.

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I. Introduction.

The variable factors which may affect the standardization of œstrin fall into four main groups: (*a*) individual variation in sensitivity of the test animals, (*b*) variation in the condition of the animals, (*c*) difference in method of administration, (*d*) difference in criteria of activity.

Individual variation. The original method of assaying œstrin was to give graduated doses to a series of animals and to consider the unit as the least amount required to produce œstrus in an ovariectomized animal. Coward and Burn(4), however, have pointed out that individual variation in sensitivity and variation in the same animal from time to time may result in a given amount being inactive in one animal and a smaller amount active in another. They thus conclude that the administration of graduated amounts is of little value for accurate assay. Coward and Burn further showed that in a large batch of animals the percentage response rose as the amount given was increased. Percentage response plotted against amount given gave the typical S-curve found by Trevan(13) to be characteristic of the reaction of batches of animals to various toxins and drugs. The curve obtained was very flat, extending from 2·5 mg. (8 p.c. on) to 17·5 mg. (84 p.c. on). This was undoubtedly due to the use of miscellaneous animals obtained from dealers. Winton(14) has shown that curves of this type are much steeper for more homozygous material. As a result of their work, Coward and Burn suggested that the unit should be defined as the amount required to bring 50 p.c. of a batch of 20 ovariectomized animals into œstrus.

Subsequently, Allan, Dickens, Dodds and Howitt(1) found that the use of 20 animals to the batch was insufficient to overcome individual variation even when very homozygous (Wistar) rats were used. These authors observed quite considerable variation in response of groups of 20 to the injection of the same amount.

We have found the same irregularity both between groups of 20 and to a much less degree in the same groups at different times.

Condition of animal. Among the more important incidental factors which might affect the sensitivity of an animal to injection of œstrin are the following: age, weight, time after ovariectomy, and previous treatment. The age cannot be supposed to exert much effect until senility. Variation in weight, however, by causing variation in amount given per grm. might well be supposed to cause variation in response. Bugbee and Simond(3) correct their results to allow for variation in weight, but Coward and Burn failed to find that this factor had any influence. The time after ovariectomy may be of importance for two reasons. Firstly, the ovariectomized animal lays on fat very rapidly, and secondly, progressive atrophy of the uterus and vagina follows the operation. Such atrophy, with the coincident decline in sensitivity, would presumably be arrested by the induction of regular œstrus periods. Another effect of the previous treatment is found in the decreased absorption of the injections which may result from subcutaneous granulations caused by the administration of bad emulsions.

Method of administration. Points of importance in this connection are:

(*a*) Site of administration.
(*b*) Medium (aqueous solution or fat suspension).
(*c*) Number of injections in which dose is given.
(*d*) Time over which injections spread.

Oral administration has been found to be most inefficient, requiring up to 100 times the dose by injection(6, 9). Intravenous injection is only becoming possible now that reasonably pure extracts are available. Intra-peritoneal injection(5) has not been found to have any advantages over subcutaneous administration, which is almost universally employed.

The stimulus producing œstrus in the normal animal is undoubtedly exerted over at least 24 hours previous to the appearance of œstrus, and in order to simulate this process in the ovariectomized animal, it is necessary to arrange for the absorption of a continuous supply of the hormone over at least a similar period. With the old fatty extracts, which were absorbed slowly over a period of days (one large dose would keep an animal in œstrus for up to 14 days), one injection would give this continuous supply. In the more recent and comparatively pure water soluble form, however, the hormone is undoubtedly absorbed and destroyed very rapidly, and to maintain a continuous supply in the ovariectomized animal for 24 hours a series of injections is necessary. Laqueur(7) and Dodds(1) and their co-workers, working with water

soluble preparations, give the dose in six injections over 48 hours. Very much larger amounts of the aqueous preparations are required to give a positive reaction when given in one dose. To give the dose of such preparations in one injection is not simulating the events in the normal animal and is physiologically unsound. The optimum time over which to spread the administration of the dose, and the optimum number of injections in which it should be given, have therefore been investigated. In addition it was thought advisable to consider the effect of volume of injection on the intensity of the response.

Criteria of œstrus. The only criterion of œstrus which need be considered here is the vaginal cornification test. This reaction has a far more definite end point than any other so far available, but much divergence is found in the application of the method. Some workers consider that the mere disappearance of leucocytes from the smear is sufficient to indicate a positive reaction (7, 8, 10), while others insist on complete cornification of the vaginal contents (1, 4, 11). The use of the pro-œstrous smear as showing a positive reaction is to be condemned on the grounds that it is much more readily confused with variations in the operative diœstrous smear than is the full œstrous smear.

Another important point is the number of observations of the vaginal contents made per day. If the period of cornified smears lasts appreciably less than 24 hours (as is often the case in the weaker reactions) daily examinations will clearly miss a number of positive results. At least 2 or 3 smears per 24 hours are necessary if pure cornified smears are not to be missed. Our own routine has been to smear three times on the critical days.

Objects of investigation. The investigation described below was undertaken in order to work out a method of assay sufficiently reliable to make possible fairly accurate quantitative chemical work on œstrin. The system finally adopted does not give the maximum value of a preparation, but it does appear to be a reasonable blend of efficiency and convenience.

II. Methods and materials.

Animals. Albino mice drawn from a colony inbred for 7 years were used exclusively. They were ovariectomized as previously described (12), except that the top of each cornu was also removed. This procedure has been found to reduce the incidence of ovarian regeneration from about 10 p.c. to 2–3 p.c.

The œstrin used was prepared from urine of pregnancy by a method based on that of Zondek and Brahn (15). Nineteen litres of fresh urine

were extracted with ether four times. The ethereal extract was washed four times with water and the residue after removal of the ether was partially saponified by heating for 10 minutes with 200 c.c. of 4 p.c. aqueous KOH on a boiling water bath. The mixture after diluting with water was extracted six times with ether and the extract washed six times with water. The residue, after evaporation of the ether, was extracted for 10 minutes with 40 c.c. of $N/10$ CH_3COOH, by vigorously shaking the flask while heating on a boiling water bath. The addition to the flask of a small quantity of silver sand washed with acid facilitated the extraction by breaking up the unsaponifiable matter. After cooling the extract was filtered through a pad of glass wool. This extraction was repeated seven times. After each extraction the residue was re-dissolved in ether, which was then evaporated again, in order to expose a fresh surface each time for extraction by the acid. The combined filtrates were carefully neutralized with NaOH to *p*H 6·8 and made up to 500 c.c. The solution was then boiled for a few minutes and 0·1 p.c. of tricresol added as a preservative. The product was water clear, and in a preliminary test proved to be highly active when administered in four doses, of 0·01 c.c. each, over 36 hours. The total solid content of the solution was 0·62 p.c., while the total ether soluble material was as low as 6 mg. p.c.

The residual unsaponifiable matter after the acid extraction was found still to contain a considerable amount of active material.

Administration. In order to investigate the effect of such variables as body weight and group response, it was necessary to fix on some arbitrary technique of administration. The fact that the growth processes in the vagina, preliminary to œstrus, appear to occupy about 36 hours, suggested that this was probably the optimum period to spread the injections over. Since this permitted four injections, 12 hours apart, without inconvenience, it was decided to adopt that procedure. The effect of the volume of fluid injected being unknown, all injections were given in a volume of 0·1 c.c., except in the experiment where this point was investigated and in the experiments on oil emulsions.

Criteria of œstrus. The appearance of vaginal smears consisting entirely of cornified cells or a mixture of nucleated and cornified cells has been accepted as indicating a positive reaction, but the presence of cornified cells and the complete absence of leucocytes have been rigidly insisted upon.

The frequency with which smears are made is a factor of considerable importance. An animal showing a weak positive response may often

give the characteristic cornified smear for a few hours only. Hence many positive reactions may be missed if the smears are only recorded every 24 hours, or even every 12 hours. Throughout this work smears have been taken at approximately 9 a.m., 4 p.m. and 11 p.m., starting 48 hours after the first injection and being continued until 48 hours after the last injection. The interval between 11 p.m. and 9 a.m. is admittedly too long and the records suggest that a few positive reactions may have been missed. However, it has been found impracticable to adopt a regular 8 hour interval as a routine method. The smears were stained with 1 p.c. aqueous methylene blue before examination to ensure the greatest possible accuracy.

III. Individual variation.

Numerous results have been obtained confirming the observations by Coward and Burn(4) that individual animals may show great variation in response to given doses of œstrin. Thus 0·27 mg. of a fatty preparation of œstrin from placenta was sufficient to produce a full œstrous response in one mouse when given in one injection, whereas 3·78 mg. failed to produce a positive reaction in another mouse injected in a similar manner.

It has also been found in many cases that the same animal may show different responses to equal doses of œstrin at different times. This is clearly shown by the records of two mice in Table I.

Table I. Variation in response of individual mice to 1·5 m.u.[1] of œstrin.

No. of mouse	Date injected	Response
O.M. 111	4. xii. 28	+
,,	28. i. 29	−
,,	12. ii. 29	−
O.M. 109	4. xii. 28	−
,,	28. i. 29	+
,,	12. ii. 29	+

[1] For the sake of clearness, the amounts of œstrin administered are expressed in terms of mouse units, in this and subsequent tables, although the unit is not defined until later.

IV. Group variation.

In order to ascertain approximately what range of variation in percentage response would be shown by different groups of 20 animals, the whole stock of mice were injected with a dose of œstrin which had been previously found to be roughly the amount necessary to produce a 50 p.c. response (1 m.u.). The results are shown in Table II.

TABLE II. Variation in response of groups of mice to 1 m.u. of œstrin.

Group	No. of mice	No. showing œstrus	P.c. response
1	20	12	60
2	20	10	50
3	20	9	45
4	18	6	33·3
5	21	11	52·4
6	19	8	42·1

This variation, although somewhat more than anticipated, did not preclude the possibility of an accurate assay, since the accuracy depends not merely upon the group variation but also upon the slope of the final standardization curve. It was hoped, therefore, that the magnitude of the group variation would prove to be a result of the steepness of the curve, in which case it would represent a comparatively small percentage error on the quantity of œstrin injected.

One would expect that similar variations would occur in any one group injected with the same quantity of œstrin at different times, but the only available data on this point, admittedly inadequate, indicates a surprising regularity in response with a dose of 1 m.u.

TABLE III. Variation of groups of mice in response to 1 m.u. of œstrin at different times.

Date injected	No. of group	No. of animals	P.c. response
3. xii. 28	3	20	45
12. ii. 29	3	20	43
3. xii. 28	6	19	42
28. i. 29	6	20	40
12. ii. 29	6	21	47·6

V. INFLUENCE OF WEIGHT AND TIME AFTER OVARIECTOMY.

Ovariectomy without subsequent treatment tends to produce a marked increase in weight, largely as the result of the deposition of fat. In our material such a relationship is obscured by (*a*) the periodic injection of œstrin, and (*b*) the fact that ovariectomy was regularly performed at 10–16 weeks of age and subsequent increase of weight was partly accounted for by increase of age. In the circumstances, therefore, statistics relative to this correlation would be of little value.

Influence of weight. Our ovariectomized animals varied in weight from 19 to 39 grm. The data available on the influence of this variation on response to œstrin are shown in Tables IV and V.

These results suggest that a slight decrease in response is found as the weight of the mouse increases, but the figures are not statistically significant, and in any case it is clear that the usual variations in weight

TABLE IV. Influence of weight of animal on response to 1 m.u. of œstrin.

Weight of animal (grm.)	No. of animals	Response +	Response −	Percentage positive
19–23	22	12	10	54·6
24–28	64	32	32	50·0
29–34	27	11	16	40·7
35–38	4	1	3	25·0
Total	117	56	61	47·8

TABLE V. Influence of weight of animal on response to 1·5 units of œstrin.

Weight of animal (grm.)	No. of animals	Response +	Response −	Percentage positive
19–23	16	14	2	87·5
24–28	55	45	10	81·9
29–34	24	15	9	62·5
34–39	3	3	0	100·0
Total	98	77	21	78·6

do not cause any disturbing variation in response to a given amount of œstrin.

The figures relating to time after ovariectomy are shown in Table VI, from which it will be seen that from 8 to 41 weeks after ovariectomy no regular variation in response to 1 unit is observed.

TABLE VI. Influence of time after ovariectomy on response to 1 unit of œstrin.

Time after ovariectomy (weeks)	No. of animals	Response +	Response −	Percentage positive
8–11	78	37	41	47·5
12–15	—	—	—	—
16–19	—	—	—	—
20–23	24	10	14	41·6
24–27	11	5	6	45·4
28–31	11	5	6	45·4
32–35	21	12	9	57·2
36–41	33	13	20	39·4
Total	178	82	96	46·1

VI. EFFECT OF PREVIOUS TREATMENT.

The continued injection of water soluble preparations of œstrin every fortnight over a period of several months had no adverse effect on the mice and no falling off in response has been observed. Thus Group 1 were injected on the 19th November, 1928, with 1·5 m.u. and 75 p.c. of the mice gave an œstrous response. On the 11th March, 1929, the same group were again injected with 1·5 m.u. and again a 75 p.c. response was observed. During the interval this group received seven injections of œstrin at fortnightly intervals.

VII. Number, volume and frequency of injections.

It is now well established that aqueous œstrin injected in a series of small injections has a greater activity than when given in a single injection of the same total amount(1, 7). It is obvious, therefore, that the number of injections any particular dose is given in, as well as the period over which they are spread, are factors of the greatest importance. Laqueur(7) and Dodds(1) and their co-workers adopt the procedure of giving a dose in six injections spread irregularly over 48 hours. This procedure can be criticized on several grounds. In the first place, it is doubtful if the increase in activity found by giving six injections instead of four is sufficient to justify the extra labour. Secondly, the irregularity of the interval between the six doses is unsatisfactory, since the object of dividing the dose is to maintain an even concentration of œstrin in the blood over a long period. Thirdly, there is no reason to suppose that 48 hours is the optimum period over which to spread the injections. On physiological grounds, it might be supposed that the period should be somewhat shorter.

Accordingly experiments were planned to investigate (*a*) the effect of a variable number of injections over a constant period, (*b*) the optimum interval between a constant number of injections.

Variation in number of injections—fat extracts. Coward and Burn, working with an oily preparation of œstrin producing a 44 p.c. response in a single injection of 10 mg., could find no increase of activity when the same total amount was given in three injections at 4-hour intervals. This was probably due to the relatively slow rate of absorption of oily œstrin compared with the purer water soluble preparations and the short total time covered by the injections.

It has, however, been found that the increase in activity shown by water soluble preparations when given in a multiple dose may also be shown by oily preparations under certain conditions. Thus 20 mice were injected with 0·64 mg. each of a cholesterol-free fat preparation from placentæ emulsified in 0·5 p.c. Na_2CO_3, and only one mouse gave a positive reaction. The same total amount was then given in four injections at 12-hour intervals to another group of 20 mice, and 12 showed a positive reaction. This experiment was then repeated with a much cruder ovarian preparation from which the cholesterol had not been removed. In a single dose of 13 mg. the response was 71 p.c.; in four doses, of 3·25 mg. each, spread over 36 hours, 100 p.c. At first sight this might appear to conflict with the generally accepted ideas on the

slow rate of absorption of crude fat extracts. However, it is more probable that the effect was due merely to a greater total absorption, since the cholesterol in the extract caused severe subcutaneous granulations, and it may be assumed that the absorption was more complete with the four smaller injections.

Variation in number of injections—aqueous extracts. The first group of mice were injected with 1 m.u. in a single injection. The second group received the same total amount of œstrin but in two equal injections with an interval of 36 hours between. The third group received the same amount in four injections evenly spaced over 36 hours; the fourth group, eight injections, and the fifth group 16 injections. Fig. 1 shows

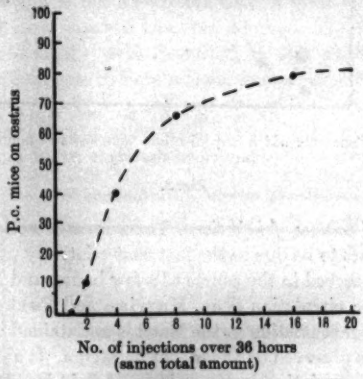

Fig. 1.

the percentage response of the different groups and demonstrates strikingly the greatly increased activity of multiple injections. The shape of the curve indicates that the response would become constant at something between 20 and 30 injections over 36 hours.

Variation of interval between injections. In order to study the effect of variation of interval between successive doses, all the groups were given 1 m.u. in four injections. The first group received the four injections simultaneously in four different places, the second group with a 2-hour interval between successive doses, the third with a 4-hour interval and so on, so that the periods over which the injections were spread were respectively, 0, 6, 12, 24, 36 and 48 hours.

The percentage response of each group is shown in Fig. 2. It will

be noticed that the highest responses are given when the interval between the injections is 8 and 12 hours, while there is a distinct loss of activity when the doses are spread over 48 hours.

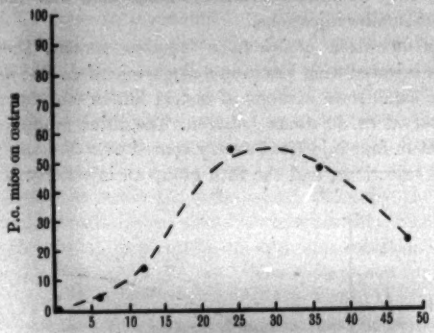

Fig. 2.

Variation of volume of injections. The increased activity of multiple injections appears to be due to the fact that relatively pure preparations of œstrin are absorbed in the course of a few hours, and are then rapidly destroyed in the circulation (Fee, Marrian and Parkes—see p. 377), and unless the concentration in the blood is maintained at the necessary level, no effect is observed on the uterus or vagina. It appeared possible, therefore, that a variation in the volume of fluid in which a particular dose was given might alter the activity by influencing the rate of absorption. To test this point two groups of mice were injected with 1 m.u. in the usual manner, with the exception that each injection was in a volume of 0·4 c.c. instead of the usual 0·1 c.c.

Table VII shows a comparison between the results obtained and the results of a large number of injections of the same amount of œstrin given in the usual volume.

Table VII. Influence of the volume of injection on the percentage response to 1 m.u. œstrin.

No. of mice used	Vol. of injection	No. on œstrus	P.c. response
160	4 × 0·1	75	46·9
41	4 × 0·4	21	51·2

This experiment clearly shows that within reasonable working limits no error is introduced by varying the total volume of fluid injected.

VIII. Method finally adopted.

It is clearly impracticable to administer a dose of œstrin in the number of injections required to give the maximum activity, and since the use of six or eight injections involves considerable inconvenience without attaining this end, it is considered that four injections is the best to adopt for routine purposes. From Fig. 2 it might appear that an 8-hour interval would give a slightly higher response than one of 12 hours. The difference, however, is well within the normal limits of group variation and is not significant, and in view of its greater convenience the 12-hour interval has been adopted. The arbitrary system used in the preliminary work described in this paper thus actually happened to approximate as close to maximum efficiency as is practicable for routine work.

The mice were then injected with varying quantities of œstrin and a standardization curve constructed (Fig. 3). The extreme steepness of

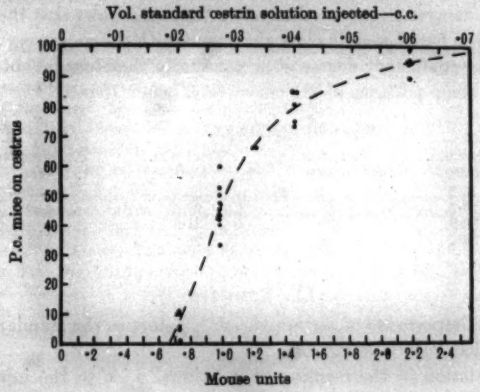

Fig. 3.

the curve near the 50 p.c. point is noticeable, and argues that the group variation does not introduce a serious experimental error.

Accuracy of the method. The probable error of the number of mice responding to a given dose may be calculated from the usual formula

$0{\cdot}6745\sqrt{\frac{pq}{N}}$, where p is the percentage of mice giving an œstrous response, q is the percentage not on œstrus and N is the number of animals used. The error found for different percentages can be translated into error on the mouse unit by reference to the curve. Table VIII shows the results so obtained.

TABLE VIII.

P.c. mice on œstrus	Probable error of p.c. mice on œstrus	Error on mouse unit p.c.
80	±6·0	+15·6 −9·6
65	±7·2	+10·3 −6·9
50	±7·6	+ 7·0 −4·0
35	±7·2	+ 4·3 −5·4
20	±6·0	+ 4·8 −5·9

The fact that the greatest accuracy is apparently obtainable just below the 50 p.c. point in the curve is of course due to the fact that the curve is steepest between the 20 and 40 p.c. points.

The accuracy which might be expected in practice was then determined experimentally in the following manner. Amounts of standardized œstrin unknown to either of us were accurately measured out and all made up to the same volume in volumetric 10 c.c. flasks. These solutions were then assayed in the usual manner. Table IX shows that the number of units thus found were in close agreement with the previous standardization. A satisfactory degree of accuracy can therefore be obtained by using the steep portions of the curve—*e.g.* below 70 p.c.

TABLE IX.

Total m.u. present	P.c. mice on œstrus	Total m.u. found	P.c. error on m.u.
18·2	14	20	+ 9·9
27·3	60	27·5	+ 0·8
30·9	71	31·5	+ 1·9
32·7	65	29	−11·3

IX. SUMMARY.

1. The importance of certain variable factors in the standardization of œstrin has been investigated.

2. Variation in the reaction of individual mice to the injection of the same amount is considerable even among fairly homozygous animals. Similar, though less, variation is found in the reaction of groups of 20.

3. The time after ovariectomy and the weight of the animal do not exert appreciable influence on the response.

4. In the case of aqueous preparations of œstrin, splitting the dose into a series of injections greatly increases the percentage response. This

is undoubtedly due to the rapid absorption and destruction of such extracts.

5. The optimum time over which injections should be spread is 24–36 hours. The percentage response increases with the number of injections in which the dose is administered over this time. Sixteen injections over 36 hours (*i.e.* injection every 2 hours 20 minutes) produce nearly twice the number of positive reactions as four injections. The volume injected does not influence the result.

6. The method finally adopted for routine testing is to give four injections at 12-hour intervals, *i.e.* to cover 36 hours.

7. Re-standardization showed an error of not more than 10 p.c.

We are much indebted to Prof. J. C. Drummond for his interest and advice, and we have been fortunate in being able to compare results with Prof. E. C. Dodds.

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A MECHANICAL STROMUHR.

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The importance of measuring the amount of blood passing through various blood vessels under different conditions has been realized almost since the beginning of the study of the circulation. The methods which are used at present for blood flow measurement can be divided into two classes, indirect methods and direct methods. The modern indirect thermo-electric methods introduced by Gesell(1) and by Rein(2) involve complicated instruments and laborious calibration. The direct methods employ some variety of stromuhr based on the principle of the Ludwig(3) stromuhr, which is sufficiently well known to need no description here. The blood flow through the Ludwig stromuhr and the Tigerstedt(4) stromuhr was reversed by turning them about a central pivot. Stolnikov(5) working in Ludwig's laboratory reversed the blood flow through his stromuhr by using a hand clamp to divert the blood through suitably connected rubber tubes. Pavlov(6) introduced an electro-magnetic clamp worked automatically by contacts operated by floats placed on the surface of the blood in the reservoirs. To make certain that each float closed its contact effectively air occupied the space in the instrument above the floats. This, of course, involved a considerable error in the measurements owing to the effect of pressure changes upon the volume of air in the stromuhr. The use of the automatic clamp, however, eliminated the subjective error in the measurements made by these hand-reversed stromuhrs. Hürthle(7) and his pupil Burton-Opitz(8) eliminated the subjective error in another way, they kept the hand reversal, and recorded the movements of a piston in the stromuhr, but this introduced an additional resistance to the blood flow. Each of these methods has a certain disadvantage.

This paper describes a new stromuhr (Fig. 1) which has been used for a series of experiments, the results of which will be published shortly. Subjective errors are avoided by the use of the Pavlov automatic electro-magnetic clamp, and the air cushion has been eliminated by the use of a special pair of contacts which are closed in air outside the instrument. They are so constructed that neither the ease with which

they are closed nor the accuracy of the measurements is affected by changes in the blood-pressure. Although the construction of these contacts is somewhat complicated their principle is simple.

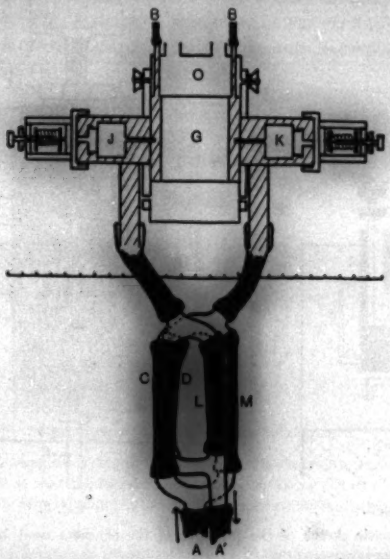

Fig. 1. Scale diagram of mechanical stromuhr. The rubber tubing and glass connections are slightly larger than their correct scale size. Scale: centimetres. Shaded area filled with blood. *A* and *A'* connected to canulæ in a divided vessel. When blood enters through *A* and the automatic clamp is set to close tubes *C* and *D*, then hollow piston *G* is moved to the left till it hits contact *J* which reverses the automatic clamp and the direction of flow till the piston closes contact *K* and so on. *O*, water bath. Tubes *B*, *B* for air escape when filling the stromuhr.

Fig. 2 is a sketch showing the principle of the contact. The rod *A* is attached to the cylinder *B* by two rubber membranes *C* one at either end of the cylinder. The inside of the cylinder is air-tight and blood surrounds the outside. The area of the two membranes is the same, hence variations in the blood-pressure cause equal and opposite differences of pressure across the membranes, and the position of the cylinder *A* is uninfluenced by changes in the blood-pressure. A stout wire *D*

attached to the cylinder *B* runs up a hole in the centre of the rod *A*. Another stout wire *G* is attached to the outside of the cylinder and

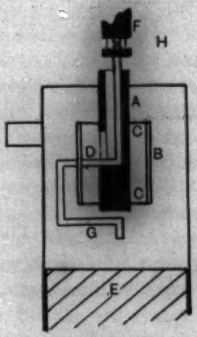

Fig. 2.

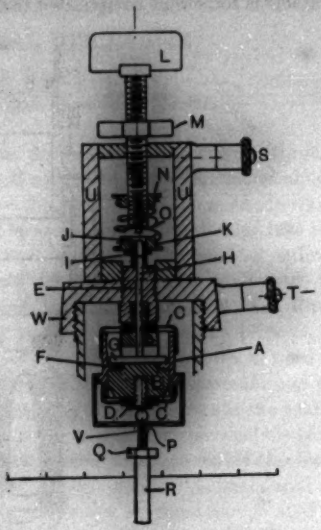

Fig. 3.

Fig. 2. Diagrammatic sketch of the principle of the contacts used in the mechanical stromuhr. See text.

Fig. 3. Scale diagram of one of the two contacts used in the mechanical stromuhr. Scale: centimetres. *A* cylinder with shoulders *F* resting on bearing *B* can move upwards only. *C* rubber membranes and washers connecting cylinder *A* to either end of bearing *B*. Bearing *B* threaded to rod *E* fixed to cap *W*. Cross-bar *G* passing through large hole in bearing *B* rests on shoulders *F*, carries light rod *H*, brass cap *I*, platinum point *J* and ebonite disc *K*, can be moved upwards only. Terminal for this contact *T*. Fixed platinum point carried by screw *L*. Terminal for this contact *S*, incalated from *T* by ebonite strips *U*. After adjusting distance between contacts screw *L* is locked by wing-nut *M*. Perforated cap *P* threaded to cylinder *A* carries threaded rod *V*, threaded tube *R* and lock-nut *Q*. Upward pressure on tube *R* causes upward movement of cylinder *A*, shoulders *F*, cross-bar *G* and light rod *H* till platinum point *J* hits corresponding fixed point on screw *L*. After the upward pressure is removed the points are separated by the light spring *O*, the tension of which is adjusted by turning the ebonite disc *N*.

projects below it. When the piston *E* moving upwards reaches *G*, the cylinder *B* and the wire *D* are moved upwards till *D* touches *F*. This

makes an electric contact in air which throws the automatic clamp into action, the direction of the blood flow is reversed, the piston is forced downwards and the contact broken by the light spring *H*.

A scale diagram of one of the contacts used in the instrument is shown in Fig. 3. Fig. 4 shows a side view of the stromuhr and clamp and a front view of the clamp. Details of the automatic clamp are given in Pavlov's paper(6).

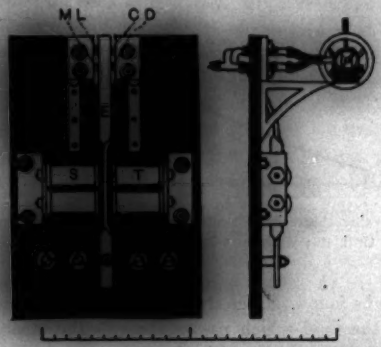

Fig. 4. Left: Electro-magnetic clamp. When magnet *S* is electrified, lever *E* clamps rubber tubes *L* and *M* so that the blood flows through the tubes *D* and *C*. Right: Electro-magnetic clamp with stromuhr in position. Side view. Scale: inches.

Resistance and accuracy of measurements.

(1) *Resistance.* A curve showing the resistance which the stromuhr opposes to different water flows is shown in Fig. 5. This low resistance is only made possible by the small friction between the piston and its cylinder. It has 1/1000″ clearance, and can be blown from one end of the cylinder to the other by a puff of air like the piston of a piston recorder. There is no leak past it.

(2) *Stroke capacity.* The velocity of the flow of a liquid through the stromuhr can be calculated from the formula

$$C = \frac{S \times 60}{T},$$

where C is the velocity of flow in c.c. per minute.

S is the quantity of fluid passing through the instrument between two reversals or, as I shall call it, the stroke capacity.

T is the time interval between two reversals.

Each reversal of the stromuhr is marked on a drum, and a time marker marks seconds on the same drum. From the records of these two

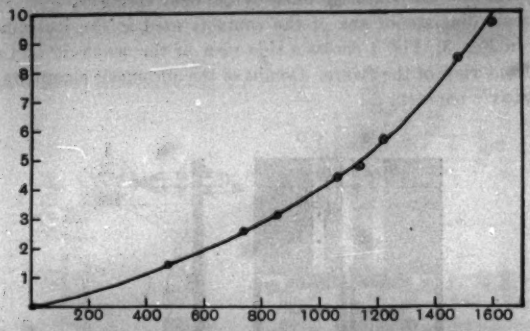

Fig. 5. Curve showing the resistance offered by the mechanical stromuhr to different water flows. Ordinate: Resistance in mm. of mercury. Abscissa: Flow in c.c. per minute.

markers T can be found. S, the stroke capacity of the stromuhr, is found by collecting the fluid which passes through the stromuhr during 50 reversals and dividing this quantity by 50.

The stroke capacity differs slightly with different flows and different pressures as the figures in the first three columns of Table I show.

TABLE I.

Water flow through the stromuhr c.c. per min.	Pressure mm. Hg	Stroke capacity	P.c. error in taking an average stroke capacity of 26·9 c.c. as a standard
138	20	25·9	3·85
126	180	26·4	1·9
352	20	26·2	2·65
308	200	26·8	0·37
741	20	27·1	0·74
615	200	27·7	2·9
994	20	27·4	1·8
933	200	27·6	2·5

These differences in the stroke capacity are not due to leak past the piston, but to the fact that the reversing of the clamp is not completely instantaneous and for an instant all four rubber tubes are open simultaneously.

The average of these stroke capacities is 26·9 c.c. If this average stroke capacity is taken as S for calculating the velocity of flow for any

one of these different flows and pressures instead of the actual stroke capacity, then there will be a small error in the calculated flow. This error is shown in the fourth column of Table I. The error is so small that it justifies the use of an average stroke capacity in calculating flows within these limits of flow and pressure.

(3) *Calibration after blood-flow measurement.* The stroke capacity of the stromuhr varies slightly each time it is taken to pieces to be cleaned and must be found for each experiment. In practice the stroke capacity used in the calculations of the velocity of the blood flow in any experiment is that found after the experiment for a water flow of 500 c.c. per minute. That it is justified is shown by the following test.

Blood has been passed through the stromuhr at a known velocity and 50 readings taken for each of 12 different conditions of flow and blood-pressure. The limits of blood flow were 195 c.c. per minute and 935 c.c. per minute, and of blood-pressure 50 mm. of Hg and 150 mm. of Hg. The average stroke capacity of the instrument has then been found by water calibration and the blood flow calculated for each of the 600 readings obtained. These 600 calculated flows when compared with the actual flows show the following results: 57 p.c. of the readings have an error of less than 3 p.c., 78 p.c. have an error of less than 5 p.c., 3 p.c. have an error of more than 9 p.c., and no error of 12 p.c. was found. Subjective errors in taking the measurements from the tracing are included in this.

(4) *Rapidity of response to changes in flow.* Fig. 6 shows the response

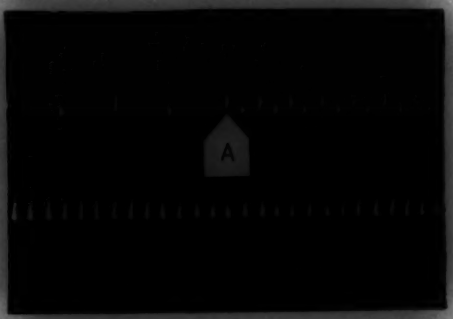

Fig. 6. Top tracing: Reversals of the stromuhr. Bottom tracing: Seconds. At *A* the constant flow through the stromuhr was instantaneously increased. This increase is recorded immediately.

of the stromuhr to a sudden change in the flow. At *A* the flow through the instrument was altered from 480 c.c. per minute to 1880 c.c. per minute. The lag in the change of the record is scarcely perceptible.

(5) *Sample tracing of blood-flow measurement.* Fig. 7 shows a sample

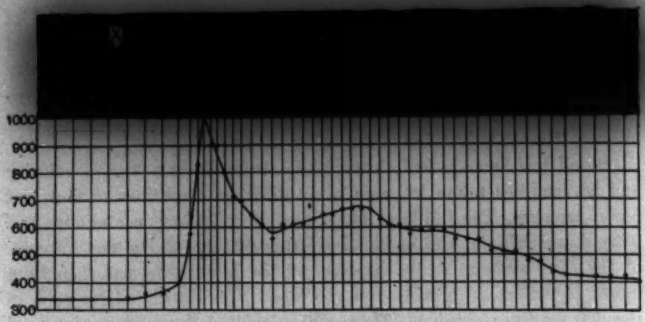

Fig. 7. Top tracing: Blood pressure. Middle tracing: Mechanical stromuhr, stroke capacity 34·5 c.c. Bottom tracing: Time marker, marking in seconds. The curve below shows the output of the heart before and after an intravenous injection of adrenaline at *X* on the tracing. Ordinate: Output of the heart (except coronary flow) in c.c. per minute. Quarter actual size.

tracing taken while the stromuhr was measuring the total output of the heart with the exception of the coronary flow, the variations in the blood-pressure and blood flow are due to an intravenous injection of adrenaline. The blood flow calculated from this tracing is shown below in the same figure.

Summary.

This paper describes a mechanical stromuhr which is suitable for the measurement of the blood flow through the larger arteries of operated animals.

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THE RELATIONSHIP OF PEPTIC ACTIVITY TO NEUTRAL CHLORIDE SECRETION DURING GASTRIC DIGESTION IN MAN.

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THE enzymes occurring in the gastric secretion have been studied for many years, but not until recently has there been any serious attempt to throw light on the variations in the peptic activity of the gastric contents during digestion. Most of the observations of gastric peptic activity have been carried out on single samples of fluid removed from the stomach at some time after ingestion of a meal, but recently there have been a few investigations by the fractional method. A variety of methods have been used for the estimation of peptic activity and there is a general lack of uniformity in the conclusions drawn by investigators.

Some workers have found a relationship between gastric acidity and peptic activity. Willcox(1) observed that the power of the gastric juice to clot milk was greater in cases of hyperacidity than in those of hypoacidity including gastric carcinoma, while in achylia the juice was without action on milk. Hirsch-Mamroth and Rindfleisch(2) found a similar connection between acidity and peptic activity in samples removed from the stomach 45 minutes after bread and water meals. Hirschberg(3) investigated a number of healthy subjects, and with few exceptions found a correspondence between acidity and ferment activity in samples removed from the stomach 45 minutes after a test-meal; 16 cases of achlorhydria were found to show peptic activity in varying degree.

Manasse(4) studied the peptic activity of the gastric contents during digestion by the fractional method and found throughout digestion a variable degree of activity which tended to rise as the acidity increased. Cases of achlorhydria, for example carcinoma, were found to show a certain degree of activity; in achylia he found no peptic activity. Cole

and Adie(5) also found that ferments were absent from the gastric fluid in true achylia; in the few cases of carcinoma with achlorhydria they investigated a secretion of pepsin was present.

By following the changes in peptic activity during digestion subsequent workers have shown that the conception of a relationship between peptic activity and acidity is not true for all samples. Matheson and Ammon(6), in a study of the effect of histamine on gastric secretion, found that the peptic activity of the gastric juice removed after injection of histamine reached a maximum in a short time and before the acidity attained its highest value. Thereafter the peptic activity was maintained at a fair height with a tendency to fall slightly. Mogena and Fernandez(7) in a similar investigation also found that the peptic activity reached its maximum earlier than the hydrochloric acid, the curve of acidity being irregular and usually not parallel with the acid curve. It was observed by Franke(8) that after an alcohol test-drink the peptic activity of the juice remained fairly steady in spite of large variations in acidity. Friedrich(9) investigated the digestive power of the gastric contents in 200 cases of gastric ulcer. He found that high values were common in the resting juice, usually higher than in subsequent samples. The peptic activity of the gastric fluid during digestion showed step-like variations and often the highest values were obtained at the end of the curve. This worker found that the digestive power and the acidity of the gastric fluid did not run parallel; often they were entirely independent the one of the other. Delhougne(10) has arrived at similar conclusions.

In the course of experiments in which acids were introduced into the stomach we observed that as the acidity of the gastric contents fell an increase in neutral chloride content occurred; further, not only did the neutral chloride rise but the peptic activity of the fluid also increased in a roughly parallel manner (MacLean and Griffiths(11)). In view of the undoubted secretion of neutral chloride by the gastric glands, we considered that investigation of the relation of the curve of digestive power to those of hydrochloric acid and neutral chloride in normal and pathological stomachs would be of interest and would possibly throw further light on gastric physiology.

As a result of our observations we are satisfied that during digestion the curve of peptic activity is related not to the curve of acidity but to that of neutral chloride. Moreover, in those cases in which hydrochloric acid is absent from the gastric secretion there is often a considerable degree of peptic activity associated with neutral chloride.

METHODS. *Estimation of chloride.* As in our previous work, chlorides were estimated by the method of Van Slyke (12).

Estimation of peptic activity. For this purpose a number of well-known methods were at our disposal, but none of them was entirely satisfactory. In our experience the method used by us for the determination of trypsin (13) is very well suited to the estimation of enzyme activity; we therefore decided to modify it with a view to its application to the estimation of pepsin. Since we published our work we have discovered that a method similar in principle but differing in detail had been described by McClure, Wetmore and Reynolds (14).

The procedure adopted by us was as follows:

The gastric fluid was mixed with a definite amount of acid egg albumin solution and incubated at 40° C. for two hours. At the end of this time the undigested protein was precipitated with trichloracetic acid and, after standing, the mixture was filtered. Trichloracetic acid in the concentration used does not precipitate proteoses and peptones, so that the filtrate contained the bulk of the products of digestion. The total nitrogen in the filtrate was estimated by the micro-Kjeldahl method of Folin and Denis (15), the amount being an index of the degree of proteolysis.

Solutions required:

(1) 2 p.c. (0·55 *N*) hydrochloric acid.
(2) Egg albumin solution. Egg white was well whisked and diluted with an equal volume of water and strained through glass wool; the total nitrogen in the solution was estimated by Kjeldahl's method. The solution was then diluted so that it contained 0·5 grm. of nitrogen per 100 c.c. The solution can be preserved for a short time with toluol. Egg white required dilution with roughly two volumes of water to give the requisite concentration of albumin.
(3) 25 p.c. solution of trichloracetic acid.
(4) Materials for Folin-Denis micro-Kjeldahl determination. The ammonia was removed into *N*/100 sulphuric acid by means of a current of air.

One c.c. of the gastric fluid (if not clear it was spun at a moderate rate for a short time) was delivered into a test-tube graduated at 10 c.c. If very acid one drop of methyl orange indicator was added and the necessary amount (predetermined by titration of the sample) of dilute NaOH was added to reduce the free acidity; great care must be taken not to render the sample alkaline in view of the sensitiveness of pepsin to alkali. 1 c.c. of 2 p.c. hydrochloric acid was added, followed by 5 c.c. of the albumin solution (containing 0·5 p.c. nitrogen), and finally the whole was made up to 10 c.c. with water.

After mixing, portions of 4 c.c. were delivered by a pipette into each of two dry test-tubes. One portion, which served as a control, was rapidly raised to the boiling point and cooled; both tubes were immediately placed in a water bath at a constant temperature of 40° C. for two hours. The tubes were stoppered with plugs of cotton wool. At the end of two hours the tubes were removed from the bath, 1·5 c.c. of 25 p.c. trichloracetic acid added and the contents thoroughly mixed. The tubes were then allowed to stand for some hours, preferably overnight.

After standing, the contents were filtered through a small Whatman No. 1 filter paper; sometimes it was necessary to re-filter the first few drops in order to obtain a clear filtrate: when much digestion had taken place it was found to be impossible to get rid of a faint turbidity in this way, but this incurs no error. The total nitrogen in 3 c.c. of the filtrate was estimated by the micro-Kjeldahl method, using *N*/100 acid and alkali, of which 25 c.c. is usually sufficient; 20 c.c. *N*/50 H_2SO_4 may be used.

The controls, if filtered, may not yield 3 c.c. of fluid; they are best centrifuged swiftly for some minutes when a perfectly clear supernatant liquid is obtained from which the

necessary amount is obtained easily. The peptic activity is expressed as the volume of *N*/100 sulphuric acid used up in the nitrogen determination.

Controls. In our experience the control values are small; when successive values in a fractional test-meal are compared it is usually found that these values tend to increase in amount as time progresses; this is because of an increase in the nitrogenous constituents of the gastric fluid.

The significance of the results obtained by this method. A comparison of the relative strength of enzyme solutions is best obtained by determining the time taken to effect equal change rather than the magnitude of change in equal time (Hedin(16), Bayliss(17)). Where a series of determinations has to be carried out as, for example, in an investigation such as the present one, such a procedure is out of the question because of the amount of work entailed. As will be seen quite satisfactory results are given by our method.

We carried out a series of experiments in order to determine the effect of dilution on the activity of a pepsin solution. Gastric juice removed from the human stomach after alcohol or wheat meal stimulus was used. Instead of using 1 c.c., various quantities of gastric fluid were employed, all other conditions of determination being rigidly adhered to.

A sample of gastric juice, obtained after a wheat meal, was taken and quantities of from 0·1 to 1·4 c.c. (Exp. 1) were put up with albumin and hydrochloric acid and estimated as described. Similar determinations were made on the juice after dilution with one and with six volumes of water (Exps. 2 and 3). Gastric juice removed after alcohol stimulus was used in Exps. 4 and 5. The results obtained are tabulated in Table I.

From these results it is clear that the degree of digestion is not proportional to the amount of enzyme solution; with increasing concentration of enzyme the digestive power tends to approach a limiting value. This relationship is of the exponential type; the digestive power is proportional not to the concentration of enzyme but to the logarithm of the concentration. If the digestive power (c.c. *N*/100 H_2SO_4) is plotted against the logarithm of the volume of juice (assumed to be proportional to the concentration of pepsin) a straight line graph is obtained. This exponential relationship is characteristic of enzyme action (Bayliss(17)).

As we are able only to compare the relative activity of different solutions of pepsin we have adhered to the volume of acid used in the nitrogen determination as an index of digestion.

TABLE I.

Vol. juice c.c.	c.c. $N/100$ H_2SO_4	Control c.c. $N/100$ H_2SO_4	Difference
Exp. 1. Gastric juice, Subject C, after wheat meal:			
0·1	9·90	2·55	7·35
0·2	13·00	3·00	10·00
0·4	17·45	3·60	13·85
0·6	20·05	4·35	15·70
0·8	22·15	4·35	17·80
1·2	22·60	5·25	17·35
1·4	21·60	5·55	16·05
Exp. 2. The same juice diluted with an equal volume of water:			
0·1	6·20	1·20	5·00
0·2	9·55	1·45	8·10
0·4	13·75	2·05	11·70
0·6	15·75	2·50	13·25
0·8	19·00	2·85	16·15
1·0	19·85	3·30	16·55
1·2	21·50	3·45	18·05
1·4	23·05	3·75	19·30
Exp. 3. The same juice diluted with six volumes of water:			
0·1	2·85	0·50	2·35
0·2	4·55	0·70	3·85
0·4	6·50	1·35	5·15
0·6	8·75	1·60	7·15
0·8	9·75	1·95	7·80
1·0	11·00	2·25	8·75
1·2	12·25	2·45	9·80
1·4	13·05	2·55	10·50
Exp. 4. Subject F, after alcohol stimulus:			
0·2	7·95	0·55	7·40
0·4	11·40	1·25	10·15
0·6	12·30	1·70	10·60
0·8	14·35	2·05	12·30
1·0	14·50	2·35	12·15
1·2	15·05	2·60	12·45
Exp. 5. Subject G, after alcohol stimulus:			
0·1	4·85	0·25	4·60
0·2	7·15	0·90	6·25
0·4	9·70	1·30	8·40
0·6	11·75	1·65	10·10
0·8	13·00	1·95	11·05
1·0	14·00	2·05	11·95

The influence of sodium chloride on peptic activity. It is well known that neutral salts in sufficient concentration lower the activity of pepsin, and it was thought that for this reason the neutral chloride of the gastric secretion might be a factor of importance in the process of digestion, since its concentration increases as the stomach empties and the need for peptic digestion diminishes.

Hamburger and Halpern(18) investigated the influence of inorganic and organic salts upon the activity of pepsin. They found that

sodium chloride in low concentrations, 0·1 p.c. (about 0·02 *N*) accelerated the rate of digestion of protein by pepsin, whereas higher concentrations, 2·5 p.c. (0·43 *N*) completely inhibited digestion; intermediate concentrations (about 0·05 *N*) had little or no effect. Clifford(19) found that sodium chloride in concentrations up to 0·26 *N* accelerated the rate of clotting of milk by pepsin. Fischer(20), using the refractometric method of estimating peptic activity, found that the activity of pepsin was materially influenced by the presence of salts; sodium chloride in concentrations ranging from 0·26 to 0·85 *N* was found to have a progressively adverse effect.

We carried out some experiments in order to determine whether sodium chloride in concentration within the range of concentration found in the stomach (up to 0·75 p.c. or 0·13 *N*) had any appreciable effect on the activity of pepsin as determined by digestion of egg albumin.

Experimental. The following experiments (Table II) were carried out with a solution containing 0·1 grm. of pepsin scales (B.P.) per 100 c.c. The digestive power of 1 c.c. of this solution in the presence of varying concentrations of hydrochloric acid and sodium chloride was estimated.

The concentrations of hydrochloric acid and sodium chloride given represent the final values after making the volumes up to 10 c.c. in the graduated tubes: the concentration of hydrochloric acid is the total acidity; the actual *p*H of the fluid was not determined. The degree of digestion is expressed in terms of c.c. of *N*/100 sulphuric acid (corrected for blank—see p. 412).

TABLE II.

HCl normality	Hydrolysis (c.c. *N*/100 H_2SO_4) No NaCl present	With 0·15 p.c. NaCl (0·026 *N*)	With 0·7 p.c. NaCl (0·12 *N*)
0·130	5·3	5·1	—
0·104	5·7	5·5	3·5
0·078	5·9	5·9	4·5
0·065	6·1	6·4	4·8
0·052	6·1	6·4	4·6
0·039	5·4	6·0	4·0
0·026	4·5	5·2	3·2
0·013	2·6	2·4	1·7
0·000	0·0	0·0	0·0

The optimum acidity for digestion with no NaCl, as shown in Col. 1, is in the neighbourhood of 0·06 *N* hydrochloric acid (0·22 p.c.); this agrees with the accepted figure for egg albumin.

Sodium chloride in low concentration (0·026 *N*) appears to increase the activity of pepsin to a small degree when the acidity of the medium

is below the optimum value; higher concentration of sodium chloride (0·12 *N*) tends to depress the activity generally.

This action is further exemplified in the following experiments (Table III); with 0·05 *N* hydrochloric acid increasing concentration of sodium chloride tends to diminish the activity of the pepsin; with 0·012 *N* hydrochloric acid there is a tendency for the activity to increase with increasing concentration of salt.

TABLE III.

NaCl normality	Hydrolysis (c.c. H_2SO_4/100)	
	With HCl 0·05 *N*	With HCl 0·012 *N*
0·104	4·2	2·8
0·078	5·0	2·8
0·052	5·0	2·8
0·026	5·6	2·7
0·013	5·7	2·6
0·007	5·5	2·6
0·000	5·0	2·3

These findings are in agreement with those of Hamburger and Halpern (18), but there is a considerable difference in degree. Clifford (19) found that the clotting of milk by pepsin was favoured by addition of sodium chloride up to 0·26 *N*, but there is some doubt as to how far the clotting of milk and the breakdown of protein are comparable processes likely to be affected in the same way by neutral salts.

Finally the digestive power of 1 c.c. of pepsin solution in the presence of sodium chloride and hydrochloric acid in concentrations such as are found in the course of a typical test-meal was determined (Table IV, Col. 3; in this table, Col. 4 shows the amount of digestion with no sodium chloride, but with the concentrations of acid shown in Col. 2).

TABLE IV.

NaCl normality	HCl normality	Hydrolysis (c.c. H_2SO_4/100)	
		HCl as in Col. 2 NaCl as in Col. 1	HCl as in Col. 2 but with no NaCl
0·015	0·017	4·8	3·8
0·019	0·028	6·4	4·6
0·036	0·049	6·0	5·3
0·057	0·064	5·4	5·5
0·079	0·035	5·1	4·5
0·103	0·020	4·7	4·5
0·103	0·024	4·9	4·2

The power of sodium chloride to increase slightly the activity of pepsin when the acidity of the medium is low is further illustrated by these results.

The solutions of albumin and pepsin used in the above experiments contained chloride, but the amount, which was constant in all the experiments, was too small to influence the results.

Perusal of the results clearly shows that peptic activity is conditioned to a far greater extent by the acidity of the medium than by the concentration of sodium chloride present, at least, when this concentration is below 0·12 *N*. As has been shown there is, however, a certain small influence to be ascribed to this salt.

We have purposely confined ourselves to concentrations of sodium chloride within the range of the gastric juice chloride; higher concentrations than this no doubt diminish peptic activity or even entirely inhibit it, but this fact can have no bearing on gastric digestion.

The curve of peptic activity during gastric digestion. The curves about to be described were obtained in the following manner: the subject was given 300 c.c. of 5 p.c. alcohol or 600 c.c. of wheat meal, and at intervals of 15 minutes samples of the gastric contents were withdrawn through a stomach tube. The samples were analysed for hydrochloric acid and neutral chloride and their peptic activity determined. The determination of peptic activity was proceeded with as rapidly as possible after removal of the fractions from the stomach to avoid the possibility of change on standing.

In the normal subject the peptic activity of the resting juice was found to be high. After ingestion of a test-meal the peptic activity of the gastric contents was found to increase progressively until the stomach was empty; the value for the peptic activity at the end of secretory activity was comparable with that of the resting juice.

Figs. 1 and 2 are representative of our findings in normal subjects after 300 c.c. of 5 p.c. alcohol; Fig. 3 after wheat meal.

It will be observed that when the gastric acidity fell the peptic activity of the contents continued to rise without interruption.

In those subjects in which gastric analysis revealed a progressive increase in gastric acidity until the end of digestion the form of the curve of peptic activity was found to be similar to that of normal individuals (Figs. 4 and 5). As before, the resting juice in these cases showed a high activity.

As will be seen from the figures, which are typical of our results as a whole, the curve of peptic activity during active secretion is similar to that of neutral chloride in so far as both curves show a progressive increase reaching a maximum towards the end of digestion. The last samples removed from the stomach resemble the resting juice in

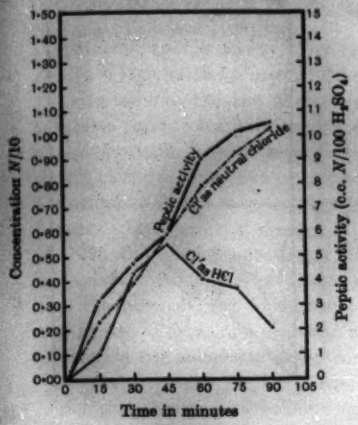

Fig. 1.

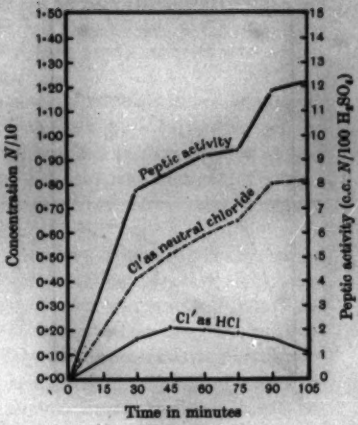

Fig. 2.

Fig. 1. Normal subject. 300 c.c., 5 p.c. alcohol. Resting juice: Cl′ as neutral chloride, 1·03 *N*/10; Cl′ as HCl, 0·17 *N*/10; peptic activity, 9·5 c.c. *N*/100 H_2SO_4.

Fig. 2. Normal subject. 300 c.c., 5 p.c. alcohol. Resting juice: Cl′ as neutral chloride, 1·00 *N*/10; peptic activity, 12·0 c.c. *N*/100 H_2SO_4.

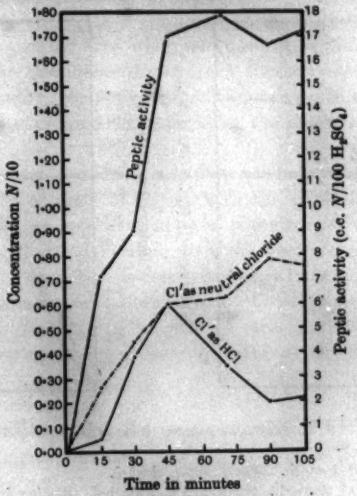

Fig. 3. Normal subject. Wheat meal.

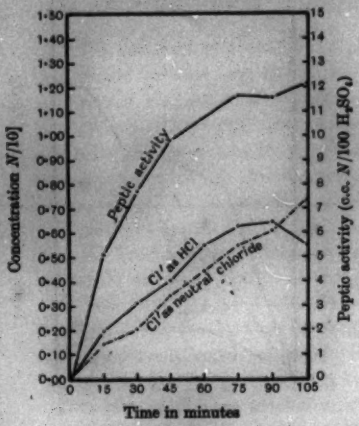

Fig. 4.

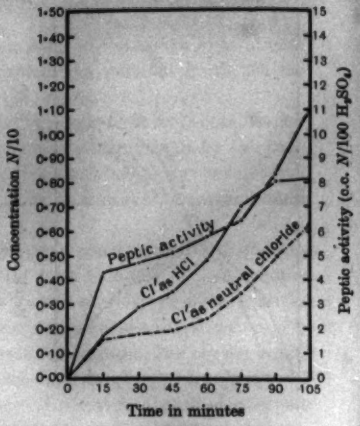

Fig. 5.

Fig. 4. Case of hyperacidity. 300 c.c., 5 p.c. alcohol. Resting juice: Cl′ as neutral chloride, 0·66 *N*/10; Cl′ as HCl, 0·34 *N*/10; peptic activity, 10·3 c.c. *N*/100 H_2SO_4.

Fig. 5. Case of hyperacidity. 300 c.c., 5 p.c. alcohol. Resting juice: Cl′ as neutral chloride, 0·68 *N*/10; Cl′ as HCl, 0·22 *N*/10; peptic activity, 9·5 c.c. *N*/100 H_2SO_4.

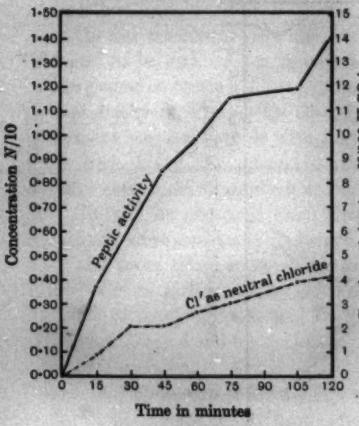

Fig. 6.

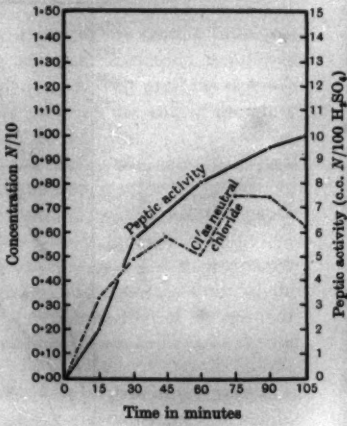

Fig. 7.

Fig. 6. Carcinoma of stomach. 300 c.c., 5 p.c. alcohol.

Fig. 7. Carcinoma of stomach. 300 c.c., 5 p.c. alcohol. Resting juice: Cl′ as neutral chloride, 0·85 *N*/10; peptic activity, 7·5 c.c. *N*/100 H_2SO_4.

character, that is to say, they have a high peptic activity associated with a high value for neutral chloride.

In view of the fact that subjects with achlorhydria are known to secrete pepsin we studied such cases with the hope of correlating peptic activity with chloride secretion. We have had an opportunity of investigating several cases of carcinoma of the stomach with achlorhydria. Such cases show a considerable secretion of neutral chloride which usually tends to be somewhat erratic.

In all such cases we found that the curve of peptic activity corresponded with the curve of neutral chloride secretion. A case of carcinoma of the stomach (Fig. 6) showed a chloride secretion reaching 0·04 *N* in 2 hours; hydrochloric acid was absent from the secretion. The neutral chloride was associated with a high degree of peptic activity.

Another case (Fig. 7) showed a somewhat higher concentration of neutral chloride (hydrochloric acid absent); from the figure it will be seen that the secretion of neutral chloride was associated with a considerable degree of peptic activity. The resting juice also had a high peptic activity accompanied by neutral chloride.

Discussion. Previous work on the peptic activity of the gastric contents during digestion has produced rather conflicting results for which, no doubt, the methods employed were largely responsible. It is important to remember that when we speak of peptic activity in connection with the present work we mean the activity under standard conditions of acidity, etc., all samples being treated in exactly the same manner; values so obtained may be called the potential activity of the gastric fluid.

Often in the past insufficient attention has been paid to the influence of acidity on the activity of pepsin, with the result that values have been recorded which represent not the potential activity of the juice but the actual activity depending upon the degree of acidity possessed by the sample. Pepsin can be estimated only in terms of its activity, and therefore, when a series of samples are to be compared with respect to their pepsin content, the activity of each sample must be determined under constant conditions in order to obtain a true comparison.

Our observations show quite clearly that after ingestion of a test-meal or after weak alcohol the peptic activity of the gastric contents increases in a regular manner, reaching its highest value towards the end of secretory activity. Moreover, the peptic activity of the juice obtained from the resting stomach has a high value comparable with

that at the end of digestion. These findings are in agreement with those of Friedrich(9) and Delhougne(10).

Cole and Adie(5), Manasse(4), Hirschberg(3) and others have pointed out that in subjects in whom hydrochloric acid is absent from the gastric secretion this fluid nevertheless shows a varying degree of peptic activity; in those cases which we have examined this observation was confirmed.

It has been stated (Willcox(1), Manasse(4), Cole and Adie(5)) that in the condition known as achylia gastrica the gastric fluid is without peptic action. It must be remembered, however, that Einhorn applied the term achylia gastrica to the particular pathological condition in which both hydrochloric acid and pepsin are absent from the gastric secretion; very frequently the meaning has been extended so as to include all those cases in which hydrochloric acid alone is lacking. There is evidence that true achylia is associated with pernicious anæmia.

After a test-meal the curves of hydrochloric acid, neutral chloride and peptic activity are found to rise, and in some cases in which the acidity of the gastric contents progressively increases to the end of secretory activity, this association of the curves is maintained until the end. In the normal type of gastric secretion, however, when the gastric acidity reaches a certain value, which varies with the individual, it begins to decline and continues to do so until the end of secretory activity. In such subjects it is found that the curves of peptic activity and neutral chloride continue to rise without interruption from the point at which the acidity begins to fall. This observation strongly suggests a connection between neutral chloride secretion and peptic activity.

If peptic activity is related in some way with neutral chloride in the gastric secretion one would expect to find a secretion of neutral chloride in those cases of achlorhydria which show some degree of peptic activity; as we have seen, such cases do exhibit a considerable degree of neutral chloride secretion. In this connection we are unable to subscribe to the opinion of Bolton(21), Miller and Smith(22) and others that the neutral chloride present in the stomach fluid in achlorhydria results from neutralization of hydrochloric acid, whether the neutralizing agent be considered to originate in the stomach or in the duodenum. In our opinion this condition is due either to a loss, on the part of the gastric glands, of the power of elaborating hydrochloric acid, or to the absence of the normal stimulus to acid secretion.

In the condition of achlorhydria all stages of transition from a normal

to a sub-normal Cl′ curve are to be found by fractional analysis. Steinitz(23) has dealt with this point at some length, basing his conclusions on a large number of cases. In the opinion of this worker and of others, the power of the stomach to elaborate acid is readily susceptible to moderate disturbances and may, from various causes, be temporarily or permanently impaired. Neutral chloride secretion, on the other hand, may persist in varying degree; definite lowering of neutral chloride secretion is to be taken as an index of grave disturbance, as in advanced carcinoma or pernicious anæmia.

In the condition of achlorhydria (with the possible exception of pernicious anæmia) the neutral chloride present in the gastric contents is associated with a considerable degree of peptic activity; in point of fact, the curves of neutral chloride and peptic activity are often similar to those of normal individuals.

As a result of these observations we must suppose that peptic activity is always associated with some degree of neutral chloride secretion, not only in the normal but also in those subjects in which there is a more or less marked pathological disturbance of the secretory mechanism.

We have shown that no physiological importance is to be attached to the effect of neutral chloride on peptic digestion; this is not the function of the neutral salt.

In our view the most probable explanation of the association of peptic activity with neutral chloride in the gastric juice is that the fluid which conveys the enzyme from the cells into the stomach cavity contains Cl′ in the form of neutral chloride.

On reflection it will be apparent that this is a reasonable supposition. Pepsin, as we know, is stored in the cells situated in the gastric mucosa; in the process of secretion the pepsin must be carried from the cells into the stomach by a fluid medium. It is this medium which is regarded as containing neutral chloride.

The increase in the peptic activity of the gastric contents which we observed after introduction of acids into the stomach (MacLean and Griffiths(11)) is therefore a normal feature of the final stage of digestion.

Conclusions.

1. After ingestion of a test-meal or dilute alcohol the peptic activity of the gastric contents progressively increases, reaching a maximum value at the end of secretory activity. The peptic activity of the resting

juice has a high value comparable with that observed at the end of digestion.

2. The curve of peptic activity is not related to the curve of acidity during digestion; a considerable degree of peptic activity may be present under conditions in which hydrochloric acid is not secreted by the stomach.

3. Peptic activity is apparently always associated with neutral chloride in the gastric secretion; the increase in neutral chloride which occurs in the gastric contents during digestion is accompanied by a corresponding increase in peptic activity so that, in a general way, peptic activity runs parallel with sodium chloride secretion.

4. Low concentrations of sodium chloride have a favourable influence on the digestion of egg albumin by pepsin, but this influence, in comparison with the influence of the reaction of the medium, does not seem to be an important one from the point of view of gastric digestion.

5. In the present state of our knowledge we must suppose that the fluid which conveys pepsin from the cells into the stomach cavity contains Cl' in the form of neutral chloride; in this way the association of peptic activity and neutral chloride secretion is explained.

These facts also substantiate our former experiments indicating the direct secretion of sodium chloride by the stomach.

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A CONVENIENT FORM OF CLOSED-CIRCUIT RESPIRATORY APPARATUS FOR MEASURING SIMULTANEOUSLY THE CARBON DIOXIDE OUTPUT AND THE OXYGEN INTAKE OVER SHORT OR LONG PERIODS.

By E. P. POULTON, W. R. SPURRELL[1] and E. C. WARNER[2].

(*From the Physiological Laboratory, Guy's Hospital.*)

In 1923 J. M. H. Campbell and E. P. Poulton demonstrated to the Physiological Society a closed-circuit respiratory apparatus, based on an apparatus described by Douglas and Haldane(1), in which direct volumetric measurement of the CO_2 output and the O_2 consumption could be made during the experimental period. The original apparatus has undergone considerable modification, and in its present form has given regular and satisfactory service for over a year. The present apparatus possesses several advantages which, we think, justify a further description.

The patient's face is fitted with a mask and valves to include the nose and mouth. The mask is of hardened rubber on a slightly malleable frame which allows of moulding to suit the subject; a thick rim of Sorbo-rubber increases the comfort of the fitting to the face. The mask is retained in place by two pairs of straps passing to a light canvas helmet; by crossing each pair of straps as in Fig. 1 the efficiency and comfort of the fitting is greatly increased.

Fig. 1.

Circuit (Fig. 2). The expiratory tube (*A*) leads to a mixing-bottle (*B*) of capacity 5000 c.c.; strips of copper wire-gauze are arranged in the bottle to promote uniform mixing. From the bottle the expired air, now thoroughly mixed, is led to a water-meter (*E*) in which is recorded the total pulmonary ventilation. Just proximal to the meter a side-tube (*D*) is tapped off leading to a portable Haldane gas-analyser in which periodical samples of the expired air are analysed for CO_2. From

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the ventilation meter a tube leads to two boxes (*F*) in series containing a mixture of soda-lime and flake caustic soda for CO_2 absorption; thence the gas is returned to the inspiratory valve. For the inspiratory and expiratory tubes connected to the valves Siebe Gorman's corrugated tubing is used, while the gas-sample tubes (*D*) and (*K*) are of fine-bore lead tubing. For convenience in prolonged use large metal containers $10 \times 10 \times 15$ ins. are employed for the boxes (*F*); these are subdivided into compartments and packed with soda-lime to afford a large absorbing surface. The tops of these boxes are covered with sheet-rubber

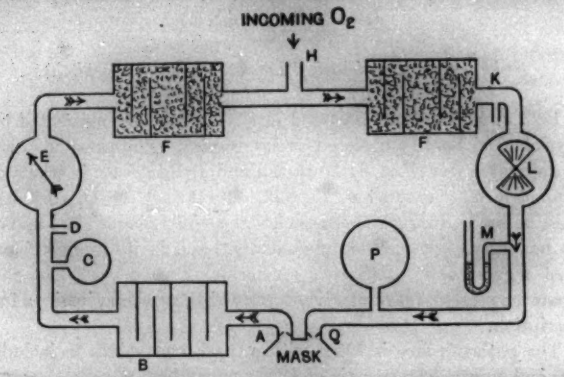

Fig. 2. *A*. Expiratory tube and valve. *B*. Mixing-bottle. *C*. Expiratory rubber balloon. *D*. Tube leading to Haldane gas-analyser for CO_2 estimation. *E*. Meter for recording the pulmonary ventilation. *F*. Soda-lime boxes. *H*. Inlet tube for O_2 supply. *K*. Tube leading to Haldane gas-analyser for the return of the residual gas after CO_2 analysis. *L*. Motor fan. *M*. Water-manometer for testing the circuit for leakage. *P*. Inspiratory rubber balloon. *Q*. Inspiratory tube and valve.

greased at the edges; over this is placed a sheet of Sorbo-rubber and finally a metal plate is clamped on, thus ensuring a gas-tight fit. Inlet and outlet tubes are brazed into the ends of the boxes, and to each outlet tube is fixed a small tube which enables periodical testing of the absorbing power of the box.

Regulation of resistance. The inclusion of a water-meter in the circuit imposes a heavy resistance upon the respiratory cycle; to minimize this load an electrically-driven fan (*L*) is included and its rate regulated to suit the needs of the subject; a rate which draws air through the meter at 7–10 litres per minute is usually sufficient for resting experiments.

Between the mixing-bottle (*B*) and the meter (*E*) is placed a small rubber balloon (*C*) of 300 c.c. capacity, which acts as an elastic reservoir preventing the subject expiring directly through the meter. Similarly, on the inspiratory side of the circuit is a larger rubber bag (*P*) of 1500 c.c. capacity, which serves as a reservoir for inspiration. The first bag serves as a useful guide to the resistance of the circuit; the fan should be so adjusted that during expiration the bag is just not completely filled.

CO_2 *estimation.* From the side-tube (*D*) a lead pipe of fine bore passes to the Haldane analyser so that samples of gas can be withdrawn and analysed for CO_2, and subsequently the residual gas is returned to the circuit by the tube (*K*). Bock and others (2) have shown that samples taken from the mixing-chamber at intervals give truly representative results as compared with the Tissot method, even in severe muscular work; we have found that the intermittent samples have given smooth results in resting subjects, and Campbell and Poulton (3) found the same thing in muscular work. The total CO_2 is calculated from the pulmonary ventilation and the CO_2 percentage.

O_2 *estimation.* As the respiratory circuit is closed and the CO_2 is all removed by the soda-lime, the circuit pressure will steadily diminish by an amount which is proportional to the amount of O_2 used up, provided conditions of temperature remain the same. O_2 is added to maintain the circuit pressure constant and the volume of O_2 added will be equal to the amount absorbed by the subject. We have found that temperature equilibrium is very soon established in the apparatus; at first it was thought necessary to immerse portions of the apparatus, such as the fan, in a water-bath, but the large cooling area afforded by the tubes, meter and mixing-chamber is adequate, as sensitive thermometers placed in various parts of the circuit indicate that equilibrium is established well within the preliminary ten minutes allowed the subject before readings are begun.

O_2 *supply.* This is shown in Fig. 3; O_2 of 99·8 p.c. purity is led into a Douglas-bag (*Z*) which acts as a reservoir; thence a tube passes to the water-trap (Müller Valve) (*R*) and dips about $\frac{1}{2}$ cm. below the level of the water; the tube (*T*) leads the O_2 from the water-trap (*R*) through the meter (*S*) into the main circuit between the caustic-soda boxes at (*H*) (Fig. 2); thus the meter (*S*) gives the O_2 consumption directly. The depth of the water-seal in (*R*) can be adjusted by the water-manometer (*Y*); for average quiet breathing it is so arranged that the O_2-meniscus lies just within the orifice of the tube (*U*) before the subject is in circuit, but if the breathing is deep and forceful, a greater depth is necessary.

The final test of the correct adjustment of the O_2 supply is found in the inspiratory bag (*P*) (Fig. 2); this should collapse at the end of each inspiration, otherwise excess O_2 will accumulate in the circuit.

In order to compensate for any possible change in pressure in the reservoir (*Z*) during the experimental period, the space above the manometer (*Y*) is connected to the Douglas-bag; thus a rise of pressure in (*Z*) which would tend to increase the supply of O_2 via the tube (*U*), would also increase the depth of the water-seal *via* the manometer (*Y*) and so the pressure conditions controlling the supply of O_2 through the water-trap would be balanced automatically. When so adjusted, the O_2 bubbles in a steady stream during the inspiratory phase only.

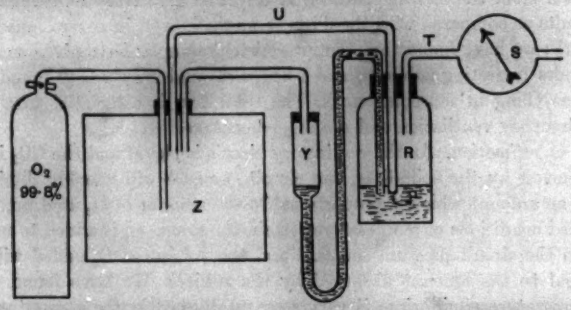

Fig. 3. *R.* Water-trap; Müller's Valve. *S.* Meter for recording O_2 intake. *T.* Tube for O_2 supply to meter. *U.* Tube for O_2 supply to Müller's Valve. *Y.* Manometer for adjusting depth of water-seal in Müller's Valve. *Z.* Douglas-bag for O_2 reservoir.

Use of the apparatus. Before use, we check the following points:

(1) Test for leakage: the mask and valves are disconnected and the O_2 inlet tube (*H*) is clamped and the pressure in the circuit raised by blowing in air or lowered by suction; the inspiratory and expiratory tubes are connected together and the water-manometer (*M*) records the pressure in the circuit; if the pressure remains steady for two minutes, the circuit is gas-tight.

(2) The motor is adjusted to transfer 10 litres per minute through the meter (*E*), or any other quantity which the experiment may require.

(3) The apparatus is then filled with O_2 by passing 15–20 litres of ordinary O_2 from a cylinder through the respiratory circuit; this is only necessary for prolonged experiments, and is to prevent N_2 accumulation owing to the slight impurity of the O_2 supply.

After any requisite preparation, the subject is fitted with the mask, care being taken to ensure accurate moulding of the face-piece; the mask and valves are then tested for leakage by getting the patient to attempt inspiration and expiration while the inlet and outlet tubes are closed with the hands. At the end of an expiration the subject is connected to the apparatus and the fan started. Ten minutes are allowed to elapse, during which period the subject settles down, temperature equilibrium is established and any little adjustments of water-seal and motor-rate made; the latter are rarely required. The O_2 and ventilation meters are read at ten-minute intervals and CO_2 analyses are made every five minutes.

The apparatus has been used for the determination of basal metabolic rates and the metabolism of various carbohydrates on resting subjects. Experiments can be carried out for several hours without discomfort, and the readings can be taken by a single observer. The accuracy of the apparatus can be tested against the Douglas-bag method, and the results agree very closely.

The following tables are examples of the consistent results given by the apparatus (Table I) and of their agreement with those obtained by the Douglas-bag method (Table II).

TABLE I. Series of consecutive determinations of basal metabolism in a single subject (case of pituitary deficiency).

Date	CO_2 c.c. per min.	O_2 c.c. per min.	R.Q.	B.M.R.	N.R.	P.c. difference
5. iv. 28	102·8	121·5	0·845	34·0	37·0	− 8·1
12. iv. 28	102·8	114·5	0·90	32·5	37·0	−12·2
14. iv. 28	102·3	118·5	0·86	33·3	37·0	−10·0
19. iv. 28	103·6	112·7	0·92	32·1	37·0	−13·2
21. iv. 28	104·7	118·0	0·89	33·4	37·0	− 9·7
28. iv. 28	98·2	110·0	0·89	31·2	37·0	−15·7
1. v. 28	103·0	113·8	0·905	32·3	37·0	−12·7

B.M.R. = Basal metabolic rate. Cals. per hour per sq. m.
N.R. = Rate as calculated from the Du Bois formula for normal subjects.

TABLE II. Comparison of results from the apparatus and from the Douglas-bag method.

Date		Apparatus	Douglas-bag
A.W. 9. xi. 27	CO_2	218·8	213·5
	O_2	257·0	258·0
	R.Q.	0·85	0·83
H.B. 10. xi. 27	CO_2	279·8	273·0
	O_2	372·5	374·0
	R.Q.	0·737	0·731
D.B. 8. xii. 27	O_2	287	290

The advantages claimed for this apparatus are:

(1) It enables direct volumetric estimation of O_2 and CO_2 to be

made; results are obtained at ten-minute intervals, and it is quite easy to get a metabolic estimation completed in half an hour; the results are obtained without disturbing the patient, and the method is also suitable for recording changes in the metabolism during a prolonged experimental period.

(2) The elimination of the gravimetric estimation of CO_2 dispenses with the need for an expensive balance, and for periodical disconnection of portions of the apparatus, with consequent risk of leakage. There is no wastage of O_2.

(3) Apart from the motor fan, the apparatus can be assembled from the usual equipment of a physiological laboratory[1]. We have mounted the whole system on a wheeled-table, so that it can be moved *en bloc* without difficulty.

SUMMARY.

A closed-circuit respiration apparatus is described, by which direct volumetric estimation of O_2 and CO_2 can be made during the experimental period. The apparatus involves no expensive additions to laboratory equipment.

The expenses of this investigation were defrayed by a grant from the Medical Research Council.

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[1] The apparatus is also supplied by Down Bros., St Thomas's Street, S.E. 1.

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PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

December 15, 1928.

The sympathetic innervation of the skeletal muscle in the wing of the pigeon: its function and morphology. By GREGOR T. POPA and FLORICA POPA. (*From the Institute of Anatomy, University College.*)

A. *Anatomy.* The ventral part of the sympathetic chain above the brachial plexus (nerves 11–14) is an elongated cervical ganglion, rich in cells and easily separated from the nerves of the brachial plexus.

B. *Removal of the sympathetic nerve supply to the wing.* From one to five thoracic ganglia immediately below the cervical ganglion were removed on one or both sides. The results confirm those of John I. Hunter and are mainly: (1) Immediate: in addition to the changes in the eye and the feathers, there is a definite drooping of the wing. (2) Remote: sensibility to cold and appetite increased. Feathers ruffled and easily plucked. Birds are dirty and do not preen themselves. Even after two months when birds are active and lively, the sympathectomised wing is easily fatigued.

The effects are always more marked as the number of ganglia removed is increased and especially after the bilateral removal. The afferent impulses from one wing may be responsible for the excess of plastic tone in the opposite wing. *Decerebration*, after sympathectomy, shows "Reflex Flying" of the wing and leg (cf. "Reflex Hanging" of Sloth).

C. *Electrical stimulation of the sympathetic trunk.* Induction coil, 50 make and break shocks per second. Excitation of the sympathetic cord between spinal nerves, 11–14, invariably produced quick contraction of the wing. Excitation of the dorsal aspect of the sympathetic cord produced depression of the neck feathers and contraction of the dorsal muscles of the neck. Excitation of the ventral aspect of the same part of the cord gave contraction of the wing and ventral muscles of the neck, with no effect on the feathers. This indicates a topographical localisation in the trunk of the sympathetic. Stimulation of the brachial plexus (or 13th spinal nerve) yielded the typical somatic contraction in the same

pigeon. Alternate stimulation of sympathetic and somatic nerves gives results which suggest a balance between the two. If the one contraction is large the other tends to be small.

D. *Pharmacological experiments.* Whereas cardiac muscle responds to atropine and ergotoxine respectively by inhibition of the parasympathetic and sympathetic, skeletal muscle contraction responds to the two drugs by inhibition and stimulation of the sympathetic. Similarly, the sympathetic contraction is diminished by K and increased by Ca, whereas the somatic contraction is increased by K, and unaffected or slightly increased by Ca. Thus in general the effects of stimulation of the sympathetic nerves is analogous to the shifting of the Ca-K balance in the direction of Ca; stimulation of the somatic nerves to shifting in the direction of K.

E. *Serological experiments.* 1. Brachial plexus and sympathetic exposed. Sympathetic stimulated for 5–10 min.; after 2–3 min. blood is collected from the aorta into 3·8 p.c. sodium citrate (2 : 1) and placed on ice-chest for 24 hours (Serum *S*). Blood, similarly treated from a normal bird, is used as a control (Serum *N*). Serum *S* and Serum *N* are injected into normal pigeons. Serum *S* causes liveliness, ruffled feathers and high position of wings, with occasional extension of the tail. The power of contraction of the wing is markedly increased. Serum *N* causes but slight increase in power.

2. *Decerebrate pigeon.* Blood collected 3 hours after decerebration and injected into normal bird produces high position of wing, general excitement and "clasp-knife" contraction.

3. (*a*) Motor and sensory roots of brachial plexus severed. Sympathetic nerves to wing excited faradically for 2–5–10 min., one minute off and on. Blood collected as above. This yields what may be called "Somatic Serum." (*b*) Sympathetic innervation of wing removed *in toto*. Somatic nerves to wing excited as in (*a*). Blood collected and yields what may be called "Sympathetic Serum."

"Somatic Serum" clears in 2–3 hours, transparent yellow, no diffusion of Hb, no buffy coat, corpuscles deposited. "Sympathetic Serum" coagulates, red colour, Hb diffuses, buffy coat most marked. Injection of "Somatic Serum" into the normal pigeon (cf. K-Ringer and atropine) yields ruffled feathers, liveliness, high position of wing, and "clasp-knife" contraction. There is frequent trembling, marked resistance to $CHCl_3$, and great danger to life in sympathetic stimulation. Injection of "Sympathetic Serum" into normal pigeon (cf. Ca-Ringer and ergotoxine) yields

depressed feathers, fatigue, listlessness, and "slow-motion" or "plastic" wing. The bird is sensitive to $CHCl_3$, and stimulation of the somatic nerves is dangerous.

F. *Histology.* Langley's account of the sympathetic in birds ignores the presence of white rami communicantes (preganglionic fibres) above the 14th spinal nerve.

Pneumin. A respiratory autacoid from the adrenal cortex discharged into the circulation via the lymphatics.

By Swale Vincent and J. H. Thompson.

(*From the Department of Physiology, Middlesex Hospital.*)

Using decerebrate cats we were desirous of investigating the vasomotor reflexes after extirpation of both adrenal bodies. We were at once confronted with the difficulty that the animals would not survive the operation for a longer period than half an hour to an hour. Death invariably occurred owing to irregularity, intermittence, and final cessation of the respiratory movements. It must be noted that cats decerebrated by our method almost invariably continue to breathe for several hours.

When respiration has ceased as described above, the animal may be kept alive indefinitely by means of artificial respiration.

Ligaturing the total blood supply to both adrenals has the same effect (somewhat delayed) as total extirpation, although ligature of the veins leaving the gland has not this effect. If damage were done to a region behind both glands, *i.e.* between them and neighbouring lymphatic glands, the same disastrous results occurred as above described, and the same is true when one removes one of these lymphatic glands in a constant position on both sides of the body. It thus became obvious that we were dealing with the lymph drainage of the adrenal glands. The next step clearly was to destroy the continuity of both lymphatic ducts. This was carried out in one experiment by cutting through the thoracic duct near the gland on the left side, and at its entrance to the subclavian vein in the neck on the right side.

Finally, we have resorted to ligature of the large veins in the neck below the entrance of the lymphatic ducts. In all these cases the result has been exactly the same as after the removal of both adrenal bodies or the removal of their accompanying lymphatic glands.

There seems no escape from the conclusion, that something is discharged from the adrenal bodies by way of the lymphatics which is

essential to normal respiration in decerebrate cats. Further it seems justifiable to assume that this material is supplied by the cortex and not the medulla of the gland; for it has been abundantly proved that it is the cortex and not the medulla which is essential to life, and in the present series of experiments extensive cauterisation of the medulla has given negative results.

Moreover, intravenous injection of fresh adrenal extract when respiration has ceased completely restores normal breathing in two or three minutes. Injections of adrenaline at this stage of respiratory inhibition fail to produce any effect and the animal rapidly dies.

Numerous controls of all kinds have been carried out. We have crushed both semi-lunar ganglia and cut all nerves in the region, severed all the connections of the adrenal bodies except in the one region indicated above (the path, we believe, of the efferent lymphatics of the cortex), and tied the veins of the gland without any results if the arterial supply be intact. These control operations have been carried out separately and collectively.

We conclude therefore that a substance which we propose to call *pneumin*, essential for the normal movements of respiration, is secreted by the adrenal cortex and passes through neighbouring lymphatic glands and into the veins by way of the thoracic duct and the right lymphatic duct.

Changes in striped muscle after sympathectomy.

By B. E. Gaissinsky and M. I. Lewantowsky.

(*Ukranian State Psycho-Neurological Institute, Charkow.*)

In a series of experiments on dogs three types of experiments were performed on the left side to indicate the effect of destruction of the sympathetic supply to the hind limb. The three operations were:

(i) Ramisection from L_1 to S_3.

(ii) Extirpation of the sympathetic trunk in the abdomen.

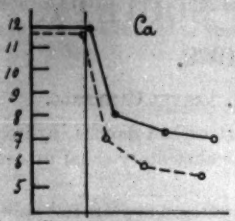
(iii) Stripping of the common iliac artery (Leriche).

In each case the blood returning from the limb deprived of the sympathetic nerve supply showed a decrease of Ca'' and Cl' and no change or a slight increase of K' and PO_4.

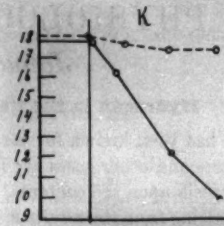
To intensify the differences, a form of "biological provocation" was employed. A preliminary parathyreoidectomy was performed as this operation produces a marked decrease in the sympathetic tone, as has been formerly demonstrated in this laboratory.

The accompanying curves show the changes in ionic concentration

in the sound and in the operated limb in the presence of the experimental tetany. The ordinates express the quantity of the ions in mgms. per 100 c.c. of serum.

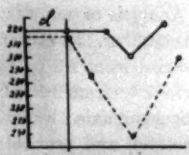


Norm. Desympathisation + Tetany.

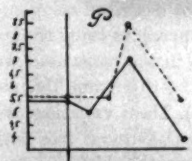


Norm. Desympathisation + Tetany.

——— right leg (normal).
- - - - - - left leg (desympathised).



Norm. Desympathisation + Tetany.



Norm. Desympathisation + Tetany.

——— right leg (normal).
- - - - - - left leg (desympathised).

Biological investigations.

Groups of dogs were subjected to:

(i) Ramisection from L_1 to S_3 on the left side.

(ii) Ramisection, extirpation of the abdominal sympathetic and stripping of the common iliac artery on the left side.

Portions of muscle were removed four months later from corresponding points in the flexor and extensor muscles of both limbs. Histological preparations were made from celloidin sections stained with hæmatoxylin-eosin and with Van Gieson. The sections show progressive loss of transverse and longitudinal striation, hyalinisation and an increase of connective tissue. The connective tissue and fat deposit are increased, particularly near the larger vessels. The processes are more advanced in the animals in which the removal of the sympathetic is the more extensive. The histological changes are degenerative and do not seem to be dependent on changes in the vascular supply. Thus the sympathetic system subserves a trophic function for skeletal muscle apart from changes in the vascular territory.

PROCEEDINGS
OF THE
PHYSIOLOGICAL SOCIETY,
January 19, 1929.

Hydrogen in the tissues. By J. ARGYLL CAMPBELL.

It has been known for many years that carbon dioxide, hydrogen, methane and other gases are formed in the alimentary canal by action of bacteria upon the contents.

Judging from the results given herein, hydrogen appears to be always present at measurable tension in the tissues surrounding the peritoneal cavity. The gas tensions were estimated by the method of injection of gas (nitrogen) described elsewhere(1). Analysis of gas was made in the Haldane apparatus using the ignition chamber. In rabbits the tension of hydrogen in the peritoneal cavity varied from 2·9 to 13·8 mm. Hg., in cats from 1·9 to 8·9 mm. Hg., and in monkeys from 2·9 to 20·7 mm. Hg. (see Table I). These variations were unaccompanied by similar changes in CO_2- and O_2-tensions (see normal results) and probably depend on several factors, the chief perhaps being rate of fermentation in the gut and rate of absorption of intestinal contents; after a few days' starvation the hydrogen disappears completely.

Care was taken to avoid puncturing the gut with the needle. The composition of gases in the gut differed strikingly from gases in the peritoneal cavity, carbon dioxide and hydrogen being present at much greater tension in the gut while oxygen was at much lower tension than in the peritoneal cavity (see Table II). The gases in the gut were obtained by anæsthetising the animal and opening the abdominal cavity.

Only a trace of carbon dioxide was formed by combustion of the gases and the oxygen used up was, within one millimeter, the same as that required to burn hydrogen. In Table I the oxygen figures are given firstly as obtained from oxygen present after ignition plus oxygen required for union with the hydrogen assumed, and secondly, the oxygen was estimated in another sample of the same gas without ignition.

Similar analyses were carried out with gas injected under the skin and the highest tension of hydrogen found was only 2 mm. Hg.; but often there was a definite trace of hydrogen and occasionally some evidence of a gas producing carbon dioxide on ignition. That hydrogen is present at much greater tension in gas in the peritoneal cavity

indicates perhaps that it reaches this region by diffusion from the lymphatics as they run under the serous membrane, the gas having been absorbed previously with other substances by the mucous membrane. Undoubtedly the gas must enter the blood capillaries also, but in this case it would be carried to the lungs rapidly and most of it excreted there.

TABLE I.

Gas tensions in peritoneal cavity, mm. Hg.

Animal	Condition	CO_2	H_2	O_2	
				Ignition	No ignition
Rabbit 23	Normal	47·3	11·5	37·9	38·0
„ 23	„	49·6	4·2	37·9	38·0
„ 23	CO exp.	34·3	9·8	26·5	27·0
„ 26	Normal	47·6	6·6	36·4	36·5
„ 26	$CO + O_2$ exp.	52·5	2·9	34·4	35·0
„ 37	Normal	47·6	6·8	41·0	41·7
„ 37	CO exp.	41·3	11·8	19·5	19·6
„ 37	$CO + O_2$ exp.	61·2	13·8	29·9	30·0
„ 32*	Normal	51·3	4·9	31·3	30·8
„ 32	CO exp.	37·0	12·1	19·8	—
„ 32	$CO + O_2$ exp.	68·1	6·6	22·1	23·0
Cat 1	Normal	47·0	8·9	28·0	26·6
„ 1	„	42·0	3·7	26·4	—
„ 1	„	37·1	1·9	33·0	32·9
Cat 2	Normal	50·1	3·2	34·1	35·0
„ 2	„	52·8	5·1	25·6	26·4
„ 2	„	52·9	3·9	26·3	27·3
Monkey 1	Normal	49·7	2·9	44·2	44·3
„ 1	„	46·2	20·7	43·1	44·1
„ 1	„	50·1	6·7	44·1	44·0
„ 2	„	48·0	9·3	34·8	35·0
„ 2	„	49·0	3·9	35·4	36·4

* Splenectomised.

TABLE II.

Comparison of gas tensions (mm. Hg.) in gut and in peritoneal cavity.

Animal	Situation	CO_2	H_2	O_2
Cat 1	Gut	94·5	21·0	10·5
	Peritoneal cavity	38·1	2·3	31·5
Cat 2	Gut	215·6	23·8	4·9
	Peritoneal cavity	50·1	3·2	35·0

Some of the results given in Table I were obtained whilst the animals were breathing mixtures of carbon monoxide and oxygen in various proportions. The tension of carbon monoxide present in the gas in the peritoneal cavity was too low to estimate by ignition; carbon monoxide was readily detected by mixing the gas with blood.

(1) Campbell. This Journ. 59. p. 1. 1924.

Gas tensions at the skin surface of man.

By J. Argyll Campbell.

With the method of injection of nitrogen into the subcutaneous tissue of man(1) it was shown that, under normal conditions, the CO_2-tension therein approximated to about 40 mm. Hg., resembling that in the alveolar air, whilst the O_2-tension was also about 40 mm. Hg. Bazett and Sribyatta(2) have recently confirmed these results using the same method.

In the present research an attempt has been made to estimate the gas tensions at the external surface of the skin. The hand, wrist, and lower part of the forearm were enclosed in a cylindrical glass chamber resembling a plethysmograph, the apparatus made gas-tight and the whole totally immersed in water at about 34° C. The cuff to close the opening surrounding the forearm was arranged so as to interfere as little as possible with the venous flow in the skin and to maintain normal skin colour.

Blank experiments indicated that the chamber was practically gas-tight when immersed in water. A thick rubber stopper, coated with solid paraffin, closed the finger-end of the chamber, the stopper being pierced by two glass tubes, one quite short and the other reaching almost to the forearm end of the chamber; by means of these two tubes the chamber was flushed out with gas mixtures of known composition. Gas samples were withdrawn from the short tube whilst an equal quantity of mercury was run in at the other tube to replace the gas withdrawn.

Table I indicates that the CO_2-tension at the skin surface must be about 40 mm. Hg. because, if lower tensions than this exist in the chamber, CO_2 comes out slowly from the skin, whilst if higher tensions than 40 mm. Hg. exist in the chamber, CO_2 goes into the skin; this tension (40 mm. Hg.) resembles the alveolar CO_2-tension.

The O_2-tensions (Table II) were not so simply elucidated. At any tension below 50 mm. Hg. O_2 fails both to go into the skin and to come out of the skin, that is at tensions lower than 50 mm. Hg. of O_2, the skin is totally impermeable to O_2. If the tension of O_2 in the chamber is much above 50 mm. Hg., O_2 then goes into the skin slowly. The skin must thus be totally impermeable to O_2 outwards or else the tension of O_2 at the skin surface must be below that necessary for permeability, that is it must be below 50 mm. Hg. The O_2-tension changes were observed in experiments where there was no great change in gas volume due to CO_2 passing into the chamber (Exps. Nos. 5, 6, 7). The capacity

of gas in the chamber was between 200–300 c.c. With air surrounding the skin about 2·5 to 3 c.c. of CO_2 come off this area every hour and about 1 to 1·5 c.c. of O_2 pass in through the same area every hour; the area of skin exposed was about 600 sq. cm. The figures for CO_2 output from the skin recall those given in the older researches.

TABLE I.

CO_2-tensions mm. Hg. in gas in chamber enclosing hand and part of forearm.

Subject	Exp. no.	At start	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours
J. A. C.	1	0	7	14	20	25	—	—	—
C. P.	2	0	6	15	23	28	32	36	38
C. P.	3	0	8	15	23	—	—	—	—
C. P.	4	33	35	36	37	39	—	—	—
C. P.	5	35	36	37	39	—	—	—	—
C. P.	6	34	35	37	40	42	43 (43)*	—	—
J. A. C.	7	37	35	36	37	38	39 (37)*	—	—
C. P.	8	46	44	43	43	—	—	—	—
J. A. C.	9	56	50	45	43	41 (41)*	—	—	—
C. P.	10	67	60	56	55	53	—	—	—

* Figures in brackets = alveolar CO_2.

TABLE II.

O_2-tensions mm. Hg. in gas in chamber enclosing hand and part of forearm.

Subject	Exp. no.	At start	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours
J. A. C.	9	10	14	12	12	12	—	—	—
C. P.	2	6	7	10	8	8	8	8	8
C. P.	5	37	36	36	36	—	—	—	—
C. P.	8	51	51	51	51	—	—	—	—
C. P.	10	53	51	50	50	49	—	—	—
J. A. C.	7	66	67	65	64	63	61	—	—
C. P.	4	86	83	78	76	73	—	—	—
J. A. C.	1	146	142	139	135	130	—	—	—
C. P.	3	147	144	140	134	—	—	—	—
C. P.	6	149	145	142	140	136	131	—	—

It seems reasonable that the skin should be permeable outwards to a waste substance like CO_2, but impermeable outwards to oxygen. That the skin appears to be permeable inwards to oxygen at the higher pressures may be due to the possibility that the softer cells near the surface depend upon the air for their oxygen supply.

(1) Campbell. This Journ. 61. p. 251. 1926.
(2) Bazett and Sribyatta. Amer. J. Physiol. 86. p. 565. 1928.

Water evaporated during work. By J. ARGYLL CAMPBELL and T. C. ANGUS.

In some experiments the subjects worked on a bicycle ergometer (12,150 kilogrammetres per hour) for six periods of twenty minutes each; in others they walked on a conveyor at the rate of 5400 metres per hour for six similar periods. The oxygen consumptions and calorific outputs during these two efforts were similar. The subjects were weighed at the beginning and end of each period, on a balance accurate to 1 gm., and the water evaporated from the body was calculated thus:

Total water evaporated = total loss of weight − (CO_2-output − O_2-intake).

The atmospheric conditions were varied to give different degrees of humidity, of temperature and of air movement.

The total water evaporated included the water lost from the skin plus the water lost from the lungs; the latter was calculated from the saturation of the inspired and expired air, with the aid of the usual formula.

At temperatures 10° C., 15° C. and 20° C., on one or two occasions the water lost from the skin was nil, the total (about 26 grm.) coming from the lungs only; at the highest temperature, 25° C., the loss from the lungs was very much less than that from the skin.

In the table are given some of the chief results for total water evaporated in relatively dry atmospheres. Subject R. usually lost much more water by evaporation than did subject P. at the higher temperatures, but the feelings of comfort did not differ greatly in the two subjects. Wind (100 to 130 metres per minute) usually reduced the amount of water evaporated, but this effect of wind was not so marked when the subjects were naked. On the whole removal of clothing reduced the total amount of water evaporated.

Pulse rates and cheek temperatures were studied as well as the water evaporated and all three were correlated with the kata-thermometer cooling-power and with the effective temperature of Yaglou and Houghten. In our numerous experiments with both resting and working subjects, the results with the kata-thermometer showed nearly as close a correlation with the physiological reactions (pulse rate, etc.) as did the effective temperature. Yaglou and Houghten have elaborated a standard of effective temperatures on equivalent sensations based entirely upon the subjective feelings of a number of "judges" who passed backwards and forwards between two rooms, one containing still, saturated air and the other air under different states of heat, humidity and move-

ment. They call the effective temperature that temperature of still, saturated air producing sensations of comfort or discomfort similar to those caused by the air in the locality under consideration. So far they have only published scales for resting and slightly active subjects, stripped to the waist and in ordinary clothing.

TABLE.

Total water evaporated from skin and lungs; grm. per hour.

	Clothed				Naked			
	On conveyor		On ergometer		On conveyor		On ergometer	
Still air T° C.	P.	R.	P.	R.	P.	R.	P.	R.
10	49	48	43	62	53	34	43	36
15	57	66	32	73	53	43	40	43
20	62	183	43	141	48	67	45	34
25	128	267	141	206	61	118	144	69
Average	74	141	65	121	54	65	68	45
Moving Air								
10	48	56	43	49	37	27	33	36
15	47	48	26	46	38	43	38	36
20	39	108	21	82	36	56	33	36
25	95	163	75	137	48	129	63	85
Average	57	94	41	79	40	64	42	48

P. 162·7 cm.; 56 kg.; 36 years of age.
R. 170·7 cm.; 64 kg.; 19 years of age.

Sphygmometry of the frog and distribution of blood-pressure.
By LEONARD HILL.

Large edible frogs were used, but the measurements can be made on the common frog. Each sphygmometer was made of (1) a rubber bag of sufficient length to surround the thigh (or tarsus), (2) a piece of thin waterproof material sufficient to overlap the rubber bag, (3) a glass tube, (4) pieces of tape. The rubber bags were made from the ends of bags sold for blowing out into sausage-shaped toy balloons. A Roy and Graham Brown apparatus for measuring capillary pressure was used for the web. This consisted of a small brass box floored with glass and roofed by a sheet of areolar tissue of the rabbit, a transparent, air-tight, and easily distended membrane. The web when stretched over this membrane was covered by a glass plate brought (by a screw adjustment) into apposition with a film of water interposed. The Roy and Graham Brown apparatus

in action is equivalent to a sphygmometer. Each of these three pieces of apparatus was connected by a T piece to a water manometer and a tube for raising pressure.

When the Roy and Graham Brown apparatus was placed on the stage of a microscope the web illuminated by a lamp could be observed with a low power. Attached to this apparatus was a sheet of cork on which lay the frog, and the two sphygmometers. The circulation in the web was observed before and after putting on the sphygmometers to make certain there was no interference through compression. The systolic pressure was ascertained by observing cessation of flow in the web.

Dr Gaddum kindly inserted for me a cannula in the sciatic artery of one thigh of a large frog, and the maximum pressure was measured directly in this by a mercurial manometer, while the arterial systolic pressure was measured in the other thigh by the sphygmometer method. The readings agreed within one millimetre of mercury. With the Roy and Graham Brown apparatus the pressure was first found which just sufficed to stop many of the loops of capillaries observed at the edge of the web. This pressure closely approximates to the capillary pressure because other capillary pathways being open the least degree of banking up is produced by the required obstruction of flow. The same pressure, or one very little higher, suffices to momentarily arrest and reverse the flow in a terminal arteriole, and when maintained to just cause a visible pulse in the flow within this arteriole which flow before was smooth and continuous. The pressure required to momentarily stop the flow in an arteriole, or produce a pulse when maintained is the true mean lateral pressure measured before banking up takes place and is about half the pressure required to keep the flow stopped.

When the flow is stopped the pressure through banking up rises to that in the artery from which the compressed arterioles arise. The conclusion is reached then that about half the head of pressure in this artery is spent in maintaining the velocity of flow in the arterioles and capillaries. In the case of the sciatic artery of the frog, when the flow is stopped the pressure banks up to that in the aorta and the systolic pressure measured is that in the aorta. In the case of the thigh sphygmometer a pressure of 18 cm. made the flow in the web pulsatile, while to stop flow there was required 36 cm. H_2O; in the case of the tarsus 13 and 27 cm.; in the case of the arterioles compressed in the web 7 and 14 cm.; to stop many capillary loops at edge of web 6 cm. sufficed; a pressure of 7 cm. H_2O sufficed to momentarily arrest and reverse the

flow in the arterioles. The capillary pressure was then in this case not higher than 6 cm. H_2O. (The above measurements are typical of many.) When a pressure of, say, 18 cm. H_2O was maintained in the thigh sphygmometer the flow became pulsatile and the web became greatly congested, and it now required 20 cm. to stop the flow in the arterioles.

T. Lewis(1) in reference to Krogh has criticised the method of measuring the capillary pressure in man by the index of the first sign of blanching of the skin, basing his criticisms on observations made when a pressure of 40 mm. Hg was maintained in a sphygmometer placed on a man's arm. The experiments on the frog show that the first sign of blanching, *i.e.* of stopping and reversing flow in some of the capillary loops, is a true index in the normal state as maintained by J. McQueen and L. Hill(2). When congestion is produced by obstruction, blanching cannot be produced until the banking up effect of the obstruction is overcome.

(1) Heart, 14. 177. 1928.
(2) This Journ. 54. Proc. cxxxiii. 1920–21.

Capillary circulation in the liver and spleen of the mouse.

By James McQueen and Leonard Hill.

J. McQueen(1) has already reported observations on the circulation in the liver of small toads; these were confirmed by L. Hill(2) who measured the pressure required to reverse the flow and found it to be 2–3 cm. of water.

The circulation in the liver of the mouse (urethaned) can be observed by making an abdominal incision just below the level of the thorax on the left, and then placing the mouse on its side so as to allow the thin edge of a lobe of the liver to fall over a glass plate which is suitably kept warm at body temperature and moistened with saline, or over the membrane covering the Roy and Graham Brown apparatus. In this case the edge of the lobe can be covered by a glass plate moistened with saline, and the pressure required to reverse the flow in the portal vein then observed under the microscope. The mouse should be small, weighing about 10 gm., and be fasted over night. In favourable cases the edge of the liver is thin enough to display almost diagrammatically a hepatic vein branching into terminal venules, portal venules between these and networks of capillaries connecting the terminal portal and hepatic venules. There is a continuous flow (rapid as seen under the microscope) through the capillaries. While in the frog there

is considerable stasis visible in the sinusoids, this is not normally the case in the mouse. Stasis comes on, however, as the mouse dies from shock. A compression of about 3 cm. of water suffices to reverse the flow and send blood up the portal venules as well as expressing blood up the hepatic venules. As the tissues of the liver are confined by a capsule, the tissue pressure must support and balance this small capillary pressure, and the conclusion is reached that there is no excess capillary pressure adequate for causing a filtration of lymph such as has been assumed.

In the case of the spleen an incision is made over this organ, and the edge of the middle of the spleen where it is thinnest is placed on the membrane of the Roy and Graham Brown apparatus and covered with the glass plate. By means of a slight compression in favourable cases, the circulation may be seen in the pulp at the edge. As a rule it is slow and scanty. This is so when the spleen is small, flat and hard. Clumps of red cells are visible together with clear parts of the pulp. A circulation may be evident here and there, blood slowly threading its way through a few channels while most of the pulp shows no movement of blood. In two cases an active circulation has been observed in a more vascular type of spleen, channels filled with moving corpuscles were seen passing through the pulp, the flow and velocity resembling that in the liver. These observations are in accord with the conclusions of J. Barcroft(3) in regard to the splenic circulation.

(1) B. M. J. Dec. 27, 1927. Aug. 7, 1928.
(2) B. J. Exp. Path. 9. 135. 1929.
(3) Die Stellung der Milz im Kreislaufsystem. Ergeb. d. Physiol. 25. 818. 1926.

The influence upon the heart of a substance present in heart muscle and other tissues. By A. N. Drury and A. Szent-Györgyi.

If freshly minced heart muscle is extracted with a hot solution of 0·25 p.c. acetic acid, or with cold 5 p.c. trichloracetic acid, the neutralised extract has a definite action upon the heart. When it is injected intravenously into the frog, dog, rabbit or cat, a transient sinus bradycardia is produced, the degree depending not only upon the amount introduced, but also upon the animal tested. In the guinea-pig the reaction differs in that the auricular rate is but little disturbed, and a transient high grade heart block occurs.

A reaction of this type lasting some ten seconds is consistently produced in different guinea-pigs by an extract from 0·5 gm. of fresh

heart muscle and subsequent injections in the same animal give reactions similar in character and degree. The occurrence of the heart block is determined electrocardiographically. By this method the relative quantities in different fractions of the substance responsible for the heart block can be determined with a high accuracy. This has allowed an attempt of purification which has led in the end to the isolation of a crystalline substance, which on recrystallisation shows a constant activity.

The substance crystallises in long needles, which melt and decompose between 190° and 200° C. It contains C, N, H, P and O. Boiled with $FeCl_3$ and HCl the substance gives a strong Kossel reaction which indicates the presence of an alloxuric base. This makes it probable that the substance is a derivative of nucleic acid. Calculating the residue as metaphosphoric acid the substance gives on combustion 32·7 p.c. C, 3·9 p.c. H, 19 p.c. N, and 13 p.c. HPO_3. This corresponds to the formula $C_{10}H_{21}N_8O_{12} - HPO_3$, which allows C and N for one purine base, one pyrimidine base and one carbohydrate.

An intravenous injection of 0·2 mg. of this substance dissolved in 1 c.c. of saline produces in the guinea-pig a transient high grade heart block similar to that produced by the simple extract of 0·4 gm. of heart muscle.

Some experiments have already been made with the crystalline product and, among others, the following results have been noted. The transient sinus bradycardia in the frog and dog and the heart block in the guinea-pig is not due to vagal stimulation as full atropinisation does not influence the result. In the dog, an injection into the femoral vein of 0·04 of a gramme slows the heart from 120 to 30 beats a minute, and full recovery is reached about 60 seconds after the injection. If auricular fibrillation is produced experimentally in the dog, this disordered action is consistently and abruptly terminated by an intravenous injection of the crystalline substance (0·04 of a gramme for a dog 6½ kilos weight), the sinus rhythm returning at a low rate and gradually rising as the effect of the injection passes away.

The substance is also to be found in the liver, spleen, kidney, striated muscle and brain, simple extracts of such tissues producing heart block in the guinea-pig.

During this investigation it has also been noted that extracts from heart muscle which have been freed from the above substance give rise on intravenous injection to ventricular fibrillation. The substance responsible for this effect is now under investigation.

An outflow recorder. By J. H. GADDUM.

It is often desirable in physiological experiments to keep a continuous record of the rate of outflow of a liquid, and various forms of apparatus have been used for this purpose. The form shown in the figure is suitable for rates of flow exceeding 10 c.cm. per minute. It is based on a principle slightly different from that usually adopted since it records the volume of liquid that flows out in a known interval of time, instead of the time taken for a known volume of liquid to flow out.

The electric circuit supplying the magnet *B* (see Fig. 1) is continually made and broken by contacts which slide on a disk partially covered with copper foil and rotated by an electric motor, the speed of which is kept constant by a governor. When the circuit is made the magnet attracts the piece of soft iron *A*, and the rubber membrane is thus made to close the end of the tube *C* for a known period of time. The volume of liquid which flows into *C* in this period is recorded by a Brodie bellows (1). The circuit is then broken and the liquid allowed to flow away, while the bellows recorder empties itself by its own weight. This process can easily be repeated every two seconds. The record thus consists of a series of upright lines, the length of each of which is a measure of the rate of flow.

The impact of the rubber membrane is liable to produce a slight movement of the recorder even when no liquid is flowing, but this movement becomes negligibly small if the membrane is made of fairly thick rubber and is loosely stretched.

If the outflowing liquid is blood, its upper surface should never rise to the top of the opening which is closed by the rubber membrane, or it will form a film. It will be seen that the plane of the opening in the tube *C* is not quite perpendicular to the axis of the tube; this is because it is found that the liquid flows in a steadier stream when the opening is made slightly oblique.

The apparatus may be calibrated either by measuring the height of the record at each of a series of different known constant rates of flow, or by calibrating the bellows separately with a syringe so that the record shows the volume of liquid flowing during the known interval in which the opening in the tube *C* is closed. It is found that these two methods give practically identical results. The calibration curve is nearly linear. The size of the record may conveniently be altered by altering the period during which the electric circuit is made. The apparatus has been used

in the perfusion of dogs' limbs and livers with blood. The advantages claimed for this type of recorder are:

(1) The record is easy to read and shows rapid changes in the rate of flow.

(2) It is not dependent on any unstable physical constant of the liquid such as its viscosity.

(3) The resistance offered to the flow need not be more than 5 mm. of water-pressure.

(4) The record may be taken on a drum at a convenient distance from the rest of the apparatus.

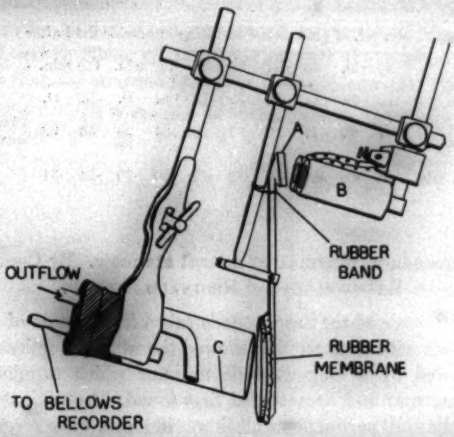


Fig. 1. When the electro-magnet *B* is activated, it attracts the piece of soft iron *A*, thus closing the end of the tube *C*. The volume of fluid flowing into *C* in this period is recorded.

(1) Brodie, This Journ. 27. 473. 1902.

Diffusion through the wall of the living and the dead intestine.

By H. E. Magee and J. J. R. Macleod.

One of us (H. E. M.) has shown, in association with Southgate, that the movements of an isolated loop of intestine (rabbit and guinea-pig) suspended in oxygenated Tyrode solution, are not immediately influenced by placing in the lumen solutions containing excess of such ions as would cause marked alterations when added to the surrounding fluid. This shows that the living intestinal wall must resist the diffusion outwards of the ions and suggested the present investigation in which

the rates of diffusion of various substances (*e.g.* sugar, potassium iodide, weak acids and dyestuffs) through the walls of living and dead loops of intestine are compared. The rates of diffusion have been measured by estimating at intervals the increase in percentage of the substance in equal quantities of Tyrode solution in which adjacent segments of gut of equal weights were suspended. One of these was alive and the other was killed by previous treatment with boiling water or sodium fluoride, and both contained equal volumes of a solution of the substance under investigation. The following table shows the nature of the results obtained for glucose, and potassium iodide.

	Mg. p.c. of glucose and of iodine in outside fluid after										
	30 min.		60 min.		90 min.		135 min.		165 min.		
Solution in lumen	L.	D.	L.	D.	L.	D.	L.	D.	L.	D.	
Glucose: 3 c.c. 5 p.c. soln.	7	27	17	55	27	80	44	95	50	92	Average of several loops
Pot. iodide: 2 c.c. 0·2 p.c. soln.	·05	·09	·08	·16	·12	·21	·20	·30	·24	·31	

The crescendo murmur of mitral stenosis. By CRIGHTON BRAMWELL(1) and REGINALD ELLIS(1).

Graphic records of the heart sounds taken from a case of developed mitral stenosis with sinus rhythm showed the usual reduplicated second sound followed by a long diastolic murmur which terminated in a crescendo murmur and accentuated first sound. The patient was then given digitalis until partial heart block was induced, when records similar to that shown in Fig. 1 were obtained.

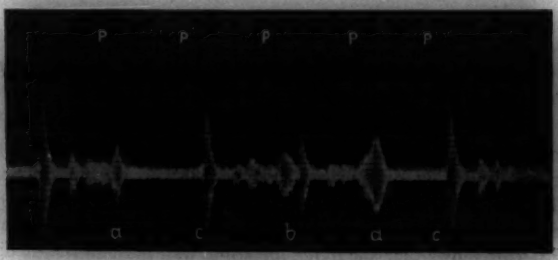


Fig. 1

These show (*a*) a loud murmur accompanying the blocked auricular beats, (*b*) a lull between the crescendo murmur and first sound in cycles where the *P-R* interval is prolonged, and (*c*) only very small vibrations preceding the first sound in cycles following the long diastolic pauses.

The most striking feature on auscultation is the loudness and long duration of the murmur accompanying the blocked auricular beats. These peculiarities may be accounted for by the fact that, following immediately on ventricular systole, the auricular contraction occurs when the ventricle is empty, and so sets up a rapid blood current between the two chambers; whereas at the end of a long diastole the high pressure in the ventricle allows only a very small movement of blood through the mitral orifice. These observations support the generally accepted hypothesis that the crescendo murmur is due to auricular systole.

(1) Working for the Medical Research Council.

PROCEEDINGS

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March 16, 1929.

A central chemical control of the heart rate.

By R. J. S. McDowall. (*King's College, London.*)

Provided adequate measures are taken to avoid the effects of sensory stimulation and of extensive operative procedures, asphyxia of the cerebral centres in a chloralosed cat, whether produced by general asphyxia, occlusion of the cerebral arteries, cerebral compression, or by supplying asphyxial blood from another animal, causes a marked acceleration of the heart. This occurs after the vagi have been divided and the suprarenal glands have been removed. The acceleration takes place prior to the better known slowing of the heart which occurs in asphyxia or cerebral anæmia from central stimulation of the vagus.

The acceleration is not related to any changes in pressure in the various blood vessels and must be looked upon as being due to the chemical effect of the asphyxia on the sympathetic. This is supported by the fact that the cardiac acceleration may be much delayed by over-ventilation of the animal prior to the production of cerebral anæmia. The central control of the heart rate may thus be somewhat similar to the control of respiration.

Observations on the respiratory exchange of hibernating mammals.

By P. A. Gorer and M. S. Pembrey. (*Physiological Laboratory, Guy's Hospital.*)

The very low respiratory quotients which have been obtained during the torpidity of hibernating mammals have been subjected to criticism. Some observers have been unable to obtain such quotients, but this failure appears to be due to the awakening of the animal during the experiment. On the other hand when such low quotients are admitted as correct findings it is maintained that they can be explained by the enhanced solubility and consequent retention of carbon dioxide in the cold body of the animal.

To meet such criticisms a series of observations has been made upon hedgehogs and upon two marmots, given by Prof. Yandell Henderson. The chief results can be condensed in the following table:

Date	H_2O grm.	CO_2 grm.	O_2 grm.	$\frac{CO_2 \text{ vol.}}{O_2 \text{ vol.}}$	T. of animal (° C.)	T. of air (° C.)	Remarks (hours)	
Marmot II								
1928								
23. x	1·79	4·15	3·34	0·90	—	13	2	Active; well fed
24. x	1·88	6·80	3·79	1·31	—	17	2	,,
15. xi	0·96	2·95	2·09	1·07	—	16	1	,,
	2·64	8·42	5·79	1·06	—	—	3	,,
1929								
8. i	0·26	1·43	1·45	0·72	10 (coil)	8·6	2	Torpid
8/9. i	2·02	3·94	4·69	0·61	—	10·5	24	,,
9/10. i	1·29	3·19	3·86	0·60	9·2 (rectum) 10 (mouth)	9	24	,,
22. ii	0·10	0·63	0·86	0·53	12 (mouth) 11 (rectum)	11·75	½	,,
Marmot I								
15. ii	0·14	0·80	1·14	0·51	10 (rectum) 13 (mouth)	5	½	,,
Marmot II								
15. i	0·34	2·41	2·60	0·67	10 (rectum) 10·5 (mouth)	9	½	Waking
	0·45	3·79	3·81	0·72	—	—	½	,,
	1·37	8·40	8·25	0·74	32·5 (rectum)	10·5	2	,,

Date	H_2O grm.	CO_2 grm.	O_2 grm.	$\frac{CO_2 \text{ vol.}}{O_2 \text{ vol.}}$	T. of animal (° C.)	T. of air (° C.)	Remarks (hours)	
Hedgehog								
1928								
29. v	3·39	2·23	2·24	0·72	35 (coil)	—	2	Active
5. xi	1·37	2·13	2·25	0·68	33·5 (coil)	13–15	2	,,
1929								
3. i	0·33	0·08	0·18	0·32	12 (coil)	9·7	2	Torpid
3/4. i	2·52	0·61	1·46	0·30	11 (coil)	10	22*	,,
12. ii	0·27	1·18	1·26	0·68	5 (coil)	3·5	3	,,
12/13. ii	2·23	1·10	1·66	0·48	—	6	24	,,
13/14. ii	1·14	1·13	1·40	0·58	5 (coil)	4	20	,,

* Wt. of animal = 494 grm.

Claude Bernard's conception of the internal environment.

By J. S. Haldane.

Prof. L. J. Henderson entitles his valuable recently published book on Blood as *A Study in General Physiology*, and at the same time treats blood as a physico-chemical system. It may escape notice that he thus makes a very far-reaching fundamental assumption; and the matter is so important that I venture to bring it before the Society. He refers to the authority of Claude Bernard in justification of his procedure; but in so doing he seems to me to have altogether misunderstood Bernard's conclusion. Bernard was the first to formulate the extremely fruitful

idea that the blood of a living animal is an internal medium kept remarkably constant as regards its physico-chemical conditions by the coordinated influence upon it of the various organs of the body. He accepts as fundamental the coordination thus displayed. L. J. Henderson, on the other hand, treats the blood as simply something which, as the result of various "buffer" reactions occurring within itself, is not as readily disturbed in its physico-chemical conditions as other liquids would be. We can, for instance, add a good deal of acid or alkali to blood without much disturbing its reaction. Or if we simultaneously add carbon dioxide and abstract oxygen from it there is a similar diminution of the disturbance which would be produced by either addition of carbon dioxide alone, or abstraction of oxygen alone.

These buffer reactions are of great importance and interest, but they were unknown to Bernard, and do not in any way modify his conception of the coordinated activity of organs by which the conditions in the blood are kept constant. This coordinated activity is an essential part of his conception of blood in the living body, whereas L. J. Henderson leaves it out of account, thus turning blood in the living body into what for a physiologist is a mere artefact, and completely disregarding Bernard's principle. It seems to me that if we disregard the coordination we have disregarded all that is characteristic of life, and that therefore the book in question cannot be regarded as a study in General Physiology, but only as a study in Physical Chemistry.

To come to details, L. J. Henderson treats the constancy of reaction in the living body as if it depended on the physico-chemical properties of blood. In actual fact this constancy depends during health on the coordinated activity of the kidneys and respiratory organs, in accordance with Bernard's principle, and in various individual parts of the body the constancy depends on the coordinated or regulated influence of the circulation. Not all the buffering in the world would keep the reaction constant otherwise, though the buffering greatly smooths the regulation. In the human body acid in excess is being continuously produced, partly as ionized sulphuric and other non-volatile acids, and partly as ionized carbonic acid. The formation of acid is constantly being exactly compensated by the excretion of acid urine and formation of ammonia on the one hand, and on the other by the washing out of carbon dioxide through the lungs. The exact coordination or regulation of these activities is the essential matter, and the quantitative investigation in various directions of physiological coordination in recent times has separated the old mechanistic physiology of last century from recent physiology.

The normal responses of the kidneys and respiratory organs depend on the simultaneous maintenance of many conditions included under the comprehensive word "health"; but we assume this maintenance in quantitative investigations of physiological function.

If, following L. J. Henderson, we neglect the coordination of organs in General Physiology, we are, it seems to me, taking a step backwards. As one who has been closely connected during the last thirty years with the development of Bernard's conception, as well as with the development of knowledge as to the physical chemistry of blood, I wish, therefore, to express my dissent from what appears to me to be Henderson's misinterpretation of Bernard. In my book, just about to be published, on *The Sciences and Philosophy*, I have discussed the subject from a wider standpoint, but before I had seen L. J. Henderson's book. It seems to me that apart from the central biological conception of specific coordination we cannot make even a beginning in the scientific treatment of General Physiology, whether we start from the unicellular organisms which Henderson unjustifiably assumes to consist of a physico-chemical system called "protoplasm," or from compound organisms with a well-defined internal environment between individual cells.

The anti-diuretic action of the separated principles of the posterior lobe of the pituitary body.

By A. Hemingway and J. M. Peterson. (*Cardiff.*)

The results of other workers (1), (2) suggest that the separated principles of the posterior lobe of the pituitary body—"Vasopressin" and "Oxytocin"—have a diuretic action when injected into the anæsthetized animal, the former being the more potent. This has been confirmed by Gaddum (3).

Whilst extracts of the whole posterior lobe have a similar action on the anæsthetized animal, they have an anti-diuretic action in arresting water diuresis, and also in the polyuria of diabetes insipidus and the excretion of urine by the perfused isolated kidney in the heart-lung-kidney preparation as described by Starling and Verney.

With a view to determining the presence or absence of this anti-diuretic principle in vasopressin and oxytocin, and, if present, its distribution between the separated principles, a series of experiments has been performed using the heart-lung-kidney technique.

From these experiments it has been found that small amounts (·05 to 0·2 c.c.) of the principles added to 1000–1500 c.c. of circulating blood are sufficient to cause marked changes in urine output and salt concentration. The principles in these strengths are without any apparent effect on the heart-lung preparation itself.

The following observations have been made:

(i) Both vasopressin and oxytocin have a prolonged anti-diuretic action.

(ii) The action of vasopressin is the more powerful.

(iii) Accompanying the anti-diuretic action there is an increase in the chloride concentration of the urine and often an increase in the total output of chloride per unit of time. This latter effect appears to depend upon the initial rate of urine flow. When this is high the total chloride output is increased; when low it is often diminished, but in each instance there is an increase in the actual concentration.

(iv) Vasopressin increases the resistance of the kidney to perfusion and diminishes the renal blood flow. This effect persists over a lengthy period.

(v) Oxytocin has only a transient effect on renal circulation, as measured by the perfusion pressure and blood flow.

1. Kamm, Aldrich, Grote, Rowe and Bugbee. Journ. Amer. Chem. Soc. 50. p. 573. 1928.
2. Bugbee and Simond. Amer. Journ. Physiol. 85. p. 357. 1928.
3. Gaddum. This Journ. 65. p. 434. 1928.

A frog's heart chamber. By B. A. McSwiney.

The apparatus illustrated in Fig. 1 was designed for experiments on the frog's heart. The chamber is made up from sheet vulcanite, the strips being cemented and screwed together. The electrodes, crystal detectors function excellently, are mounted on a movable strip of vulcanite which may be fixed in any given position by the screw shown in Fig. 1. Fine adjustment is made by movement of the electrodes.

To record the contractions of the heart an aluminium lever fitted with a frontal glass pointer is used. The lever is held by a thread suspension, and the position of the lever adjusted by altering the position of the screws, *A*, through which the cotton is threaded. The frontal writing point is easily made and has been found very suitable in practice. A fine

glass pointer is threaded through a narrow glass cuff. The pointer is bent in the flame, so that one arm is pressed against the drum paper. The cuff is attached to the lever by Chatterton cement. The chamber may be lined with sheet cork.

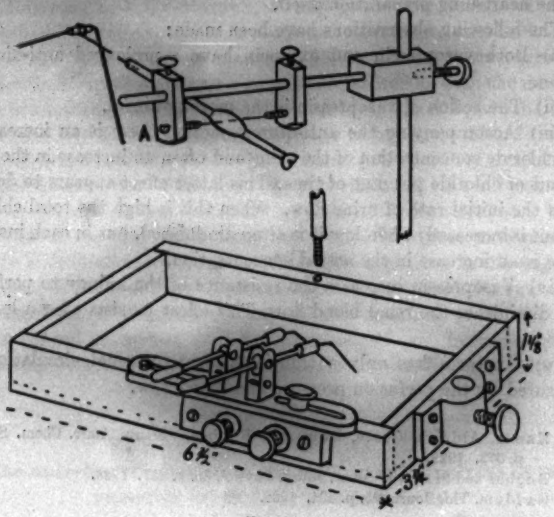

Fig. 1.

PROCEEDINGS

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Emotional disturbances following experimental lesions of the base of the brain (pre-chiasmal).

By J. F. FULTON and F. D. INGRAHAM[1] (*Oxford*).

The following experiments were undertaken on the supposition that the frontal lobes give rise to tracts of fibres which pass to the centres in the hypothalamus. The existence of such tracts was suggested by clinical evidence and by the fact that pupillary changes are produced by faradic stimulation of a discrete point on the antero-medial surface of the frontal lobes (1). The surgical procedure carried out was evolved in an effort to sever this postulated pathway and thus to release the hypothalamus from the control exerted by the frontal lobes.

We propose to describe the results obtained in four animals which survived for periods of one to three months and which were studied with care both before and after operation.

Operation. After a mid-line skin incision and reflection of the temporal muscles, generous openings were made on both sides of the skull, so that, after free resection of the dura, one could readily displace the brain by gentle retraction and thus expose the optic nerves. With a hooked knife an incision 2 to 3 mm. deep was then made 3 to 4 mm. anterior to the chiasm, extending from mid-line to olfactory radiation; after making a similar lesion on the opposite side, the operative wound was then carefully closed in layers with fine silk sutures.

Cat I. A healthy adult castrated male cat was subjected to operation in two stages. After a unilateral lesion there was no essential change in its behaviour. Following incision of the other side seven weeks after the first operation there was a marked change in the disposition of the animal. Previously friendly and playful, it settled into a state of chronic anger aroused to an intense pitch by any friendly advances. Gentle stroking of the animal's back stirred it to a fury of scratching, spitting, and attempting to bite. This condition persisted until the animal was sacrificed five weeks later, and at necropsy both lesions were found to extend to the mid-line.

[1] Peter Bent Brigham Hospital Travelling Fellow in Surgery.

Cat II. A healthy young female cat, not quite full grown, friendly and playful. A few hours after recovery from anæsthesia this animal displayed a marked reaction of rage which has persisted until the present time (11 weeks). When one reaches toward her she growls, very much as does an angry dog; if one touches her head she hisses, spits, and attempts to scratch and bite, and exhibits evidence of a diffuse sympathetic discharge, *e.g.* dilatation of pupils, sweating of feet. If one persists in stroking her she is soon aroused to a state of fury difficult to control. In other respects the animal behaves normally, *i.e.* eats well, walks normally, and is intelligent enough to tip over a dish to obtain meat hidden under it.

Cat III. A large white angora castrated male cat. Although it was impossible to elicit the slightest evidence of anger in this animal before operation, a rage response is now given upon the slightest stimulation. Any restraint causes it to scratch and bite, whereas it formerly only purred under the same circumstances. There is also a marked pilo-motor response and extreme dilatation of the pupils during rage.

Cat IV. A large friendly male tiger cat was operated upon twice, one side being done first and later the other, but after neither procedure was there any evidence of change of character. At necropsy two weeks after the second operation it was found that the lesions had not extended to the mid-line as we had intended.

It still remains to study histologically the degeneration produced by these lesions. It seems evident to us, however, from gross examination at operation and necropsy that the change of disposition noted in the first three animals was caused by the small operative lesions, and that to be effective the lesions must be bilateral. It is probable, then, that cortico-hypothalamic tracts have been divided, thus releasing the hypothalamus and so inducing a state of chronic rage, *i.e.* a condition in which rage is much more easily elicited than in a normal animal. Associated with the rage there was evidence of diffuse discharge of the sympathetic nervous system. We believe that these observations may throw light upon the emotional disturbances and changes in behaviour seen clinically with lesions of the base of the brain.

1. Karplus and Kreidl. Pflüger's Arch. 135. p. 412. 1910.

The contractile duality of the skeletal muscle.

By FRÉDÉRIC BREMER (*Brussels*).

The fundamental identity of the neuro-muscular mechanisms of the phasic and tonic contractions does not in itself exclude the possibility that the skeletal muscles of Vertebrates, at least of some of them, in addition to their ordinary quick contractility, are endowed with properties allied to those of the smooth muscles, and having another signification than their, erroneously supposed, tonic function.

The phenomenon which I have described under the name of *neuro-muscular contracture* (1) affords a new evidence of the existence of a slow contractile mechanism in amphibian skeletal muscles, and moreover it shows the possibility of its activation by nervous impulses.

Fig. 1. Neuro-muscular contracture. Decerebrated brown frog. Sciatic-gastrocnemius *in situ*. Isotonic records (5 grm. weight). The simple twitches provoked by a single maximal break shock on the nerve are marked by a point under the myogram. The double fused twitches in answer to a succession of two such maximal break shocks are each marked by a number indicating, in sigmas, the interval of the two stimuli. $T°$: 13° C. Time in seconds. The two rows of myograms have been taken from the same muscle at one hour interval. Reduction of $\frac{1}{3}$.

Note that the secondary contraction appears in each case the interval of $2,7\sigma$ (absolute refractory period of the nerve) increases up to an optimal interval of $\pm 5\sigma$, and disappears when the interval reaches $\pm 8\sigma$.

It is a slow (3 seconds' duration and more) secondary contraction (Fig. 1) of the gastrocnemius and other muscles of the brown frog and of the toad, provoked by the appropriate *summation* (*addition latente*) *of two or more nervous impulses.*

There is a close parallelism between the aptitude of a given frog muscle to neuro-muscular contracture and its aptitude to galvanic, alkaloidæ and fatigue contractures.

The necessity of a summation of nervous impulses for the elicitation of the slow secondary contraction is explained by the demonstrable existence of a considerable heterochronism (in Lapicque's sense of this term) between the slow contractile "substance" and the ordinary motor nerve fibres by which it is activated: to its slow contractility corresponds a very slow excitability. Therefore nervous impulses, as in the case of brief electrical stimuli exciting the muscle *directly*, must be summated in order to provoke the slow secondary contraction (neuro-muscular contracture).

This mechanism gives a satisfactory explanation of the pharmacological properties of the neuro-muscular contracture and specially its elective sensibility to fatigue and to the alkaloids of the atropine group.

Although the degree of shortening caused by the slow secondary contraction may exceed, and sometimes considerably, the degree of shortening of the fused twitch which precedes it, the contractile tension it develops is always much smaller than the twitch tension.

Neuro-muscular contracture has striking similitudes of properties with the so-called *myotonic rigidity* of Thomsen disease and other allied conditions.

The functional significance, if it has any, of the contractile duality of the skeletal muscles of Amphibians, and also probably of Reptiles and Birds if we judge from their reactions to nicotine and acetycholine (2), is not evident. It has probably no relation with postural tone. Perhaps it is only a vestigial character, a remnant of the primitive condition of the striated muscle fibre, recapitulated elsewhere in the muscular ontogeny, which character decreases in the vertebrate phylum, and has disappeared in the normal mammalian muscle, but may reappear in muscular diseases (myotonic conditions, degeneration).

The particular interest, besides the physio-pathological facts it explains, of neuro-muscular contracture lies in its production by a summation of nervous impulses. The facts it has revealed, confirming other summation experiments on lightly curarized or fatigued nerve-muscle preparations (3), support the Lapicque-Lillie hypothesis that the skeletal muscle fibres are excited by the action currents of the nerve.

1. Bremer. C. R. de la Soc. de Biol. 98. pp. 601, 607, 612, 1264. 1928; 100. p. 205. 1929.
2. Dale and Gasser. Journ. of Pharm. and Exp. Therapeutics, 28. p. 287. 1926; 29. p. 53. 1926.
3. Bremer. C. R. de la Soc. de Biol. 92. p. 1179. 1927.

The mechanical and electrical response of the frog's heart.

By J. Yule Bogue and Rafael Mendez (*Edinburgh*).

There has been considerable controversy as to whether substances which depress the mechanical response of the frog's ventricle cause a depression of similar extent in the electrical response.

We have tested the action of depressant agencies on the mechanical and electrical responses of the frog's ventricle.

A strip of frog's ventricle was used, to which a piece of auricle and sinus was left attached to provide regular stimuli. The mechanical response was measured with a light isometric lever, and when the electrical response was measured the deflection of the string was always standardized.

The depressants tested were: lack of calcium chloride, acetyl choline (10^{-6} molar), excess of potassium chloride (4×10^{-3} molar), acid ringer (*p*H 6·0), and octyl alcohol (2×10^{-3} molar).

These fell into three groups:

(1) Lack of calcium almost completely abolished the mechanical response without producing any measurable change in the extent or form of the electrical. (This confirms Mines(1), Daly and Clark(2) and Willigen(3), but is opposed to the results of Einthoven(4) and Arbeiter(5).)

(2) Acetyl choline did not affect the height or the duration of the "R" wave, even when the mechanical response was reduced to about 2 p.c. of the normal. The "T" wave, however, was increased in amplitude and reversed.

(3) Excess of potassium chloride, acid and octyl alcohol caused obvious changes in the form of the electrical response as soon as they produced a well-marked depression of the mechanical response. As regards the effects of excess of potassium chloride our results agree with those of Daly and Clark(2), but are opposed to the results of Einthoven and Hugenholtz(6), and Arbeiter(5), who found that KCl excess and calcium lack produced similar effects on the electrical response.

The most obvious changes noted in group (3) were a reduction in the rate of rise of the "R" wave and a fusion of the "R" and "T" waves.

We wish to take this opportunity of expressing our thanks to Prof. A. J. Clark for his help.

1. Mines, G. P. Journ. Physiol. 46. p. 188. 1913.
2. Daly, I. de B. and Clark, A. J. Journ. Physiol. 54. p. 367. 1921.
3. Willigen, A. v. D. Onder. Physiol. Utrecht, 6. p. 170. 1926.
4. Einthoven, W. Harvey Lectures, 20. p. 111. 1925.
5. Arbeiter, W. C. A. Arch. Neerl. 5. p. 185. 1921.
6. Einthoven, W. and Hugenholtz, F. W. N. Arch. Neerl. 5. p. 176. 1921.

The physiological properties of anterior lobe pituitary extract in relation to the ovary. By C. W. Bellerby.

Recent work on the relation of the anterior lobe of the pituitary to the ovary has yielded contradictory results. Whilst transplantation of the gland results in the maturation of abnormal numbers of follicles and superovulation, injection of certain extracts is followed by intensive lutealization of the ovary. These divergent results have led to the assumption that the anterior lobe elaborates two hormones which act on the ovary in different ways. Both these effects can, however, be obtained by one extract of the gland. A method for preparing extracts which yields favourable results is as follows:

The anterior lobes are dissected out, weighed, and ground to a paste with sand. The material is then extracted with twice its weight of 1 p.c. acetic acid for 24 hours at 37°. The solid matter is filtered off, the filtrate thoroughly shaken with kaolin, again filtered, and the extract then neutralized with 50 p.c. NaOH (salmon pink to phenol red). On standing over night at 0°, a precipitate comes down which is filtered off. The filtrate is used for injection without further treatment, and is preserved under a layer of paraffin.

These clear yellow extracts are not toxic and can be given intravenously in large doses (10 c.c.) without ill effects. The changes in the ovary produced as a result of subcutaneous injection (5–10 c.c. for 4 days) of this and other extracts are as follows:

(*a*). In the immature female rabbit the infantile ovaries are converted into masses of follicles, blood follicles and corpora lutea. Sexual maturity is produced, changes taking place in the uterus which do not occur until puberty.

(*b*) In the mature female rabbit two effects are produced. The follicles already existing in the ovary are transformed into corpora lutea, and at the same time the formation of so many fresh follicles is induced that the ovaries ultimately consist almost exclusively of follicles in different stages of development. With the exception of the luteal tissue and the septa of connective tissue separating the follicles there is almost complete absence of stroma (Fig. 1). Mating of these animals is followed by wholesale formation of corpora lutea.

(*c*) If the injected females are not mated, the formation of large numbers of corpora lutea still takes place. Normal atrophy of the follicles produced as a result of the injections does not occur, since they develop, without rupture, into healthy functional corpora lutea. In

some cases, however, evidence of rupture is found. Pseudo-pregnant changes take place in the uterus and in the mammary glands.

Fig. 1.

Experiments carried out on some 50 animals show that the effect of injection of extracts of the anterior lobe of pituitary is to produce not only maturation of abnormal numbers of follicles, but also their lutealization. It is not essential to postulate the existence of two hormones acting on the ovary, nor is the follicular effect dependent on "suspended" anterior lobe substance, as has been suggested.

The relation of the anterior lobe of the pituitary to ovulation.

By C. W. Bellerby.

Ovulation and the formation of the corpus luteum in the rabbit only normally occurs after copulation. No satisfactory explanation has so far been given of the mechanism involved. Recent work, however, seems to show that it may be, in part, hormonic.

During investigations carried out on the influence of extracts of the anterior lobe of the pituitary on follicular development (described elsewhere) it was observed that in several cases rupture had occurred of follicles formed as a result of subcutaneous injection although the animals had been kept in separate cages away from the male. These observations suggested that the anterior lobe might quite possibly be a factor in ovulation.

It is known that spontaneous ovulation can occur in the rabbit as a result of sexual excitement. Furthermore it was quite probable that in consequence of the abnormal production of follicles, nymphomania had been produced in the injected animals and that during the excessive

sexual excitement, these animals had ovulated spontaneously. A direct action of the extract on the follicles could not be proved in consequence.

The difficulty was overcome by making use of the facts that anæsthesia does not interfere with the ovulation process (1) and that extracts could be administered intravenously. By carrying out the whole of the experiment under anæsthesia the possibility of spontaneous ovulation occurring as a result of psychical stimuli was rendered very remote.

It was then found, by giving single injections of extract intravenously, that ovulation followed at approximately the same time after the injection as after copulation. The preparation of extracts has been described in a separate communication. A typical experiment is briefly described below.

1.5 p.m. Animal anæsthetized with urethane and ether.
1.10 p.m. Laparotomy performed. Right ovary contained five flat pink follicles. Left ovary contained three flat pink follicles.
1.20 p.m. Injected 10 c.c. of extract intravenously.
11.30 p.m. Animal anæsthetized with ether. Opened. All follicles clear, yellow and protruding definitely from surface.
12.45 a.m. Red specks appear on surface of follicles. Two show definite pimples.
12.50 a.m. Two follicles rupture in right ovary.
1.0 a.m. Third follicle ruptures in right ovary. One ruptures in left.
1.5 a.m. Second follicle ruptures in left ovary.
1.10 a.m. Extensive hæmorrhage in other follicles but no rupture.
1.30 a.m. No change in ovaries.
2.0 a.m. No change. Animal killed.

Ovulation therefore occurred in this animal eleven hours thirty minutes after injection.

It is suggested that the act of copulation or the orgasm resulting from it stimulates the anterior lobe of the pituitary to secrete into the blood stream a hormone that initiates in the follicle those internal changes that finally result in its rupture.

1. Walton and Hammond. Brit. Journ. Exp. Biol. 6. p. 190. 1928.

Spontaneous deciduomata in the rat.

By J. R. M. Innes and C. W. Bellerby.

One of us (1) has shown that pseudo-pregnancy periods of two or three weeks can be produced in the rat by single injections of anterior lobe pituitary extract. The end of many of these periods was marked by a discharge of blood from the vagina, which seemed possibly to be due to pseudo-pregnant degeneration of the uterine mucosa.

Post-mortem examination of animals killed as soon as the appearance of blood was observed showed that pronounced swellings were present in the uterine horns.

Histological examination proved these to be identical in structure with the deciduomata produced in normal pseudo-pregnancy by injury to the uterine mucosa (*i.e.* insertion of threads). No pseudo-pregnant degeneration of the uterus was observed; the uterine mucosa, apart from the swellings, showing the normal structure of pseudo-pregnancy. Hæmorrhage of these growths occurring as a result of necrosis was the direct cause of the appearance of blood in the uterus; a blood-stained vaginal smear being the only external sign. Only in sections taken below the level of these growths was blood observed in the lumen of the uterus.

Although it has been observed that the same abnormalities in the uteri of rats arise as a result of Vitamin A and E deficiency, this factor cannot be held responsible for the cases produced above; not only were the injected rats themselves assured of an adequate supply of these vitamins, but others in the colony on an identical diet showed no divergence from the normal as regards the regularity of their cycles or their breeding behaviour.

If it is assumed that two factors are essential for the formation of deciduomata, one chemical, the other an irritant, it is clear that the only irritation in the above cases can come from the ova. As other workers have apparently excluded the ovum as an irritant it seems that the chemical factor alone can produce these growths.

It is suggested that the luteal tissue produced in the ovaries as a result of injection of anterior lobe extract is abnormally active in producing a dysgenic hyperplasia of the uterus, during pseudo-pregnancy.

1. Bellerby. Lancet, ii. p. 1168. 1928.

The influence upon the heart of substances related to nucleic acid. By A. Szent-Györgyi and A. N. Drury.

We have already reported (*J. Physiol.* 67. xiv) that extracts of heart muscle and other tissues have a definite action upon the heart, and that a substance, responsible for this action, can be isolated from such extracts in crystalline form. The elementary analysis of the crystalline substance has suggested that it is composed of an alloxuric and a pyrimidine base, a carbohydrate and phosphoric acid. Additional analysis has shown that the carbohydrate is probably a pentose and the alloxuric base, adenine.

Injected into the guinea-pig this substance produces a transient high grade heart block, and substances related to nucleic acid have been

tested for the presence or absence of a similar reaction. Animal and yeast nucleic acids are inactive. The hydrolysate from a neutral hydrolysis of yeast nucleic acid at 115° C. for 2½ hours has only a slight activity, the nucleotides thus obtained being apparently inactive; but the hydrolysate from a neutral hydrolysis at 145° C. for 2½ hours is extremely active, producing transient high grade heart block similar to that seen with the substance originally isolated from heart muscle. Guanosine isolated from such a hydrolysate is inactive. The fact that the hydrolysate is readily rendered inactive by boiling with acid shows that the pyrimidine nucleosides are also inactive.

The active constituent has been isolated as a picrate, which, judged from its solubility and crystalline structure, is adenosine picrate. The activity of adenosine liberated from the picrate is of the same order as the substance isolated from heart muscle. Boiling with acid readily destroys the activity of adenosine, showing that adenine itself is inactive. Prof. G. Embden has kindly supplied us with samples of muscle adenylic acid and inosinic acid, and we find that the former produces heart block in the guinea-pig similar to that produced by the substance isolated by us, while the latter has no action. From these experiments it is evident that the part of the molecule of the various substances tested, responsible for heart block in the guinea-pig, is the adenine carbohydrate compound. Experiments are now in progress to determine the action of adenosine upon the dog's heart, etc.; they indicate that it will prove identical with the action of the substance isolated by us from heart muscle. Embden considers that the earliest change in muscle activity is the deamination of adenylic to inosinic acid. It is interesting to note also that, as judged by its action upon cardiac function, a highly active substance is converted by deamination into an inactive substance.

Further evidence for the existence of depressor fibres in sensory nerves. By I. Marcu (*Bucarest*).

(*From the Physiological Laboratory, Oxford.*)

Evidence for the existence of depressor reflexes in response to stimulation of sensory nerves has been brought forward by other workers (1, 2). The present communication deals with some experiments confirming their conclusions. The observations described here were made incidentally during experiments performed for the study of the rebound phenomenon of an extensor muscle of the hind limb. Decerebrated cats were used, and over two hours were allowed to elapse after the decerebration. The blood-

pressure was recorded in the femoral artery. The rebound contraction described by Sherrington was elicited by faradic stimulation of an afferent nerve on the same side as the dissected muscle. In one of the experiments in which the hamstring nerve was stimulated I noticed on the blood-pressure tracing a faithful copy of the rebound phenomenon taking place in the muscle. The blood-pressure rose abruptly on cessation of the stimulation. Stimulation of another afferent nerve in the same animal, the popliteal nerve, produced the same effect, although for a

Fig. 1. Effect of stimulation of the central end of the peroneal nerve on the arterial blood-pressure. Note the after effect of the stimulus.

Fig. 2. Effect of stimulation of the central end of the splanchnic nerve. Note the effect and after effect of stimulus on the arterial blood-pressure.

given strength of stimulus the magnitude of the response was not constant. Looking through a large number of blood-pressure records obtained on different animals I could find two more instances in which the same effect was obtained. Fig. 1 shows the after-rise in two cases of stimulation of the peroneal nerve with different strengths of stimuli. Both the initial rise of blood-pressure during the stimulation and the after-rises seem to be proportional to the strength of the stimulus. In these experiments the suprarenal glands were denervated, though this

is not a significant factor because in another cat with the splanchnic nerves intact stimulation of the popliteal nerve had the same effect. In this animal after cutting the splanchnic nerve its central end was stimulated with the result shown in Fig. 2. During the stimulation the blood-pressure rose in two steps, a plateau was then established and the heart slowed; after the end of stimulation the blood-pressure rose still further and then gradually fell to normal. In other experiments the same events took place without any slowing of the heart. The after-rise does not show any specificity as it was observed with stimulation of various afferent nerves. As regards the conditions determining its incidental occurrence I am at present unable to define them precisely. As it stands this after-rise suggests that during the stimulation of an afferent nerve a depressor influence tends to counterbalance the pressor effect. There is a similarity between this phenomenon and the after-rise following stimulation of the peripheral end of the splanchnic nerve(3) and considered as evidence for the presence of vaso-dilator fibres in this nerve.

1. Martin, E. G. and Mendenhall, W. L. Amer. Journ. Physiol. 38. p. 98. 1915.
2. Ranson, S. W. and von Hess, C. L. Ibid. 38. p. 128. 1915.
3. Thompson, J. H. This Journ. 65. p. 441. 1928.

PROCEEDINGS

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May 25, 1929.

Differences of structure between the white and red muscles of the rabbit. By MAY L. CAMERON, *Physiology Department, Edinburgh University.*

Adductor magnus and vastus internus (white) and semitendinosus (red) are compared. The red muscle fibres are more bluntly polygonal, sometimes larger, sometimes smaller; their nuclei much more numerous; the sarcoplasm is greater in amount. In cross sections of red muscle deep nuclei are seen in addition to peripheral nuclei but are rare; such nuclei are generally single, sometimes multiple, two or three being seen at the same sectional level. The blood supply of red muscle is richer than that of white. As is well known the transverse capillaries have actual dilatations on their course; and the longitudinal capillaries are tortuous with an irregular lumen. Red muscle appears to have a larger amount of fat in its areolar tissue, and to be more richly supplied with nerve endings. Muscle spindles are frequent in red muscle, rarer in white.

A method of determining free and bound water. By W. W. TAYLOR, *Physiology Department, Edinburgh University.*

The lowering of the opalescence-temperature of a phenol-water mixture (*e.g.* 10–11 per cent. of phenol) by the addition of known amounts of a solution is determined in a Beckmann freezing-point apparatus; the amounts of water alone which produce the same depressions are also ascertained. The latter gives the "free" water in the corresponding solutions, and the difference between it and the total water gives the amount of "bound" water.

By altering the percentage of phenol the temperature range can be varied from 68° downwards. The method is applicable to electrolytes, non-electrolytes, and to certain colloids at least, *e.g.* gum arabic, dextrin, etc. Other similar systems can be employed instead of phenol-water.

Relationship between the liver cells and the blood. By E. SHARPEY-SCHAFER, *Physiology Department, Edinburgh University.*

To judge by the accounts given in textbooks and in recent articles on the liver, the relationship between the liver cells and the blood is by no means understood either by anatomists, physiologists, or pathologists. The blood channels in the liver lobules are still alluded to as "capillaries," whereas they have nothing in common with capillaries. They are interstitial spaces, as the study of their development shows, and were rightly distinguished from capillaries by the term "sinusoids" given them by C. S. Minot. Especially it is to be noted that there is no endothelium between the blood and liver cells: the cells are directly bathed by blood.

It is generally thought that the Kupffer cells represent endothelial remains, but the evidence for this is not strong. They are similar to the phagocytic cells in the spleen, bone-marrow, and lymph-glands, and are included with these in the "reticulo-endothelial system" of Aschoff. They may have been brought to the liver in the circulating blood, for all these phagocytic cells have a tendency to settle down in places where the blood stream is sluggish. In any case, they do not line the sinusoids, nor are they intimately bound to the liver cells, for they are easily detached and pass into the blood stream; this happens when they are subjected to certain forms of stimulation, such as injection of finely ground quartz, and the like.

Another fact that has generally been ignored is that the sinusoids communicate with fine anastomosing canaliculi in the interior of the liver cells. The liver of a freshly killed animal, injected from the portal vein by carmine gelatine, shows unmistakable evidence of the direct passage of the injection into the cells: it appears within them in well-defined passages, which anastomose with one another. These passages have nothing to do with the biliary canaliculi, although in cases of obstruction of the bile ducts they may show evidence of the presence of bile, as the blood itself does. They can be seen in uninjected preparations as clear branching spaces. The accompanying photographs, magnified 975 diameters, show their relations plainly. In Fig. 1 four cells are shown exhibiting intracellular canaliculi occupied by injection. In Fig. 2 the connection between the injection mass in the sinusoids and that in the intracellular canaliculi can be seen. There is no trace of endothelium lining the sinusoids, the walls of these being formed by the liver cells themselves. The injection mass, having undergone shrinkage from the alcohol which was used to fix the preparation, has in most places shrunk

away from the wall and ruptured the communications with the intracellular canaliculi, but indications of the communications are everywhere visible. It will be seen that the Kupffer cells have become entangled in the shrunken injection mass and detached from the wall of the sinusoids.

Fig. 1.

Fig. 2.

Histological evidence of secretion by the kidney tubules of the skate. By P. T. Herring.

Sections of the kidney tubules of the skate—*Raia batis*—are shown which indicate the presence inside them of globules of an amorphous material which is evidently a secretion by the cells lining the tubules. In many places the material can be seen in the cells and different stages occurring in its extrusion by them into the tubules are illustrated. The appearances in these sections are such that one must assume that it is secretion into the tubule and not absorption by the tubule which was taking place at the time the kidney was fixed.

An investigation of the pulmonary circulation of the guinea-pig. *Preliminary report.* By G. Harold Ettinger (*Kingston, Ont.*).

From the Physiology Department, Edinburgh University.

In view of the peculiar moniliform structure of the pulmonary artery of the guinea-pig, in which there are alternations of thick musculature with parts where the muscular coat is very thin and even absent, it seemed of interest to investigate the blood flow through the lung of that animal. This investigation has been carried on partly by a method of

perfusion (with Tyrode solution) of the pulmonary vessels after death, partly by experiments on the pulmonary pressure of the living animal. The investigation has yielded the following results.

1. Adrenaline. This constricts the pulmonary arteries of male and non-pregnant female animals: moderately during August and September, powerfully during April and May, but not at all from October to March. There is evidently a seasonable variability, which requires further investigation by experiments extending over another year.

2. Extract of posterior lobe of pituitary body. The extracts used were the "pituitrin" of Parke Davis and Co., and "pitressin" of the same firm. The first dose of "pituitrin" causes contraction, a second either contraction or relaxation. "Pitressin" acts similarly, but less powerfully.

3. Barium. This always causes powerful contraction.

4. Amyl nitrite. This always causes relaxation.

5. Janus green. Minute doses cause powerful contraction. This effect is observed on perfusing both pulmonary and aortic systems, and not only in the guinea-pig, but also in the cat and rabbit.

6. The pulmonary arteries of a guinea-pig sensitized to foreign protein (horse serum, egg albumen) contract strongly on the addition of a small quantity of the same protein to the perfusate. The bronchial musculature reacts similarly and very powerfully.

The expenses of this investigation have in part been met by a grant from the Earl of Moray Fund.

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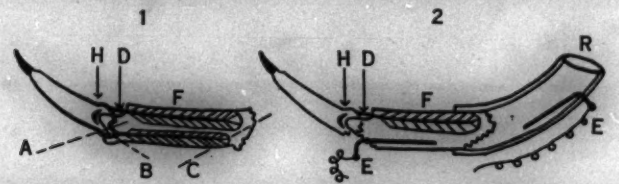
A crustacean muscle preparation. By C. F. A. PANTIN.

A good muscle preparation can be obtained from the leg of the Spider Crab, *Maia squinado*. The terminal joint hinges (*H*) on two processes on either side of the penultimate joint. There is a flexor and an extensor muscle, and these are attached over the inner surface of the penultimate joint. Each is inserted on to an endosternite: that (*D*) of the flexor muscle (*F*) is attached to the terminal joint above the hinge (*H*), and that of the extensor is attached to a chitinous knob below the hinge (1). By cutting through this knob at *A*, the endosternite of the extensor muscle can be grasped and the muscle pulled out of its seating. This leaves a simple flexor preparation (2).

Since a small movement of the muscle causes a large excursion of the terminal joint, isometric contractions are easily obtained.

The preparation is perfused through a rubber tube (*R*) into which is inserted the penultimate joint. It is advisable to cut away the chitin



along the lines *B* and *C* to allow free perfusion of the fluid over the flexor muscle. Stimulation is effected through electrodes (*E*) inserted into the extensor muscle cavity and into the perfusion tube.

The muscle is not easily stimulated to give maximal contractions with single shocks, unless these are so strong that the muscle becomes rapidly damaged. The most satisfactory stimulus is provided by a small A.C. transformer (as used for electric bells) from the mains (220 v. 50 cycle), giving a secondary E.M.F. of 3–8 volts. The transformer is connected to a potentiometer (50 ohms) from which is obtained the

desired intensity of stimulus. When stimulated thus once a minute to give a tetanus for 5 seconds, continual good responses have been obtained for as long as 6 hours.

The blood of Crustacea closely resembles sea-water in inorganic composition except that it contains less magnesium. Sea-water provides a good perfusion fluid.

The preparation is being employed to determine the effect of different ions on the contractile apparatus and on the excitability of the muscle. It provides successful class experiments to illustrate the close relation between the composition of sea-water and the serum in all groups of animals; the general similarity in response to ions between muscles from animals of different Phyla: and to interpret the action of ions on the Crustacean heart.

During the months of July and August the preparation is apt to become rapidly inexcitable. The reason for this has not yet been determined.